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(54) CELL/TISSUE CULTURING DEVICE AND METHOD

ZELL/GEWEBEKULTURSYSTEM UND -VERFAHREN

DISPOSITIF DE MISE EN CULTURE DE CELLULES/TISSUS ET SON PROCEDE D'UTILISATION

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(73) Proprietor: **Metabogal Ltd.**
11013 Kiryat Shemona (IL)

(72) Inventor: **SHAALTIEL, Yoseph**
12255 Beit Hillel (IL)

(74) Representative: **Barlow, Roy James**
J.A. KEMP & CO.
14, South Square
Gray's Inn
London WC1R 5JJ (GB)

(56) References cited:
EP-A- 0 200 792
EP-A- 0 343 885
EP-A- 0 350 723
WO-A-88/00234
DE-A- 2 654 725
GB-A- 1 053 848
GB-A- 2 202 549
US-A- 2 836 434
US-A- 4 668 632

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Description

Field of Invention

[0001] The present invention relates to devices for axenically culturing and harvesting cells and/or tissues, including bioreactors and fermentors. In particular this invention relates to such devices which are disposable but which nevertheless may be used continuously for a plurality of consecutive culturing/harvesting cycles prior to disposal of same. This invention also relates to batteries of such devices which may be used for large-scale production of cells and tissues.

Background

[0002] Cell and tissue culture techniques have been available for many years and are well known in the art. The prospect of using such culturing techniques economically is for the extraction of secondary metabolites, such as pharmaceutically active compounds, various substances to be used in cosmetics, hormones, enzymes, proteins, antigens, food additives and natural pesticides, from a harvest of the cultured cells or tissues. While potentially lucrative, this prospect has nevertheless not effectively crystallised with industrial scale bioreactors which use slow growing plant and animal cultures because of the high capital costs involved.

[0003] Prior art technology for the production of cell and/or tissue culture at industrial scale, to be used for the production of such materials, is based on glass bioreactors and stainless steel bioreactors, which are expensive capital items. Furthermore, these types of industrial bioreactors comprise complicated and expensive mixing technologies such as impellers powered through expensive and complicated sterile seals; some expensive fermentors comprise an airlift multipart construction. Successful operation of these bioreactors often require the implementation of aeration technologies which constantly need to be improved. In addition, such bioreactors are sized according to the peak volume capacity that is required at the time. Thus, problems arise when scaling up from pilot plant fermentors to large scale fermentors, or when the need arises to increase production beyond the capacity of existing bioreactors. The alternative to a large-capacity bioreactor, namely to provide a number of smaller glass or stainless steel bioreactors whose total volume capacity matches requirements, while offering a degree of flexibility for increasing or reducing overall capacity, is nevertheless much more expensive than the provision of a single larger bioreactor. Furthermore, running costs associated with most glass and stainless steel bioreactors are also high, due to low yields coupled to the need for sterilising the bioreactors after every culturing cycle. Consequently, the products extracted from cells or tissues grown in such bioreactors are expensive, and cannot at present compete commercially with comparable products produced with alternative techniques. In fact, only one Japanese company is known to use the aforementioned cell/tissue culture technique commercially, using stainless steel bioreactors. This company produces Shikonin, a compound which is used almost exclusively in Japan.

[0004] Industrial scale, and even large scale, bioreactor devices are traditionally permanent or semi-permanent components, and no disclosure nor suggestion of the concept of a disposable bioreactor device for solving the aforementioned problems regarding large scale cell/tissue culture production is known of. On the contrary, disposable fermentors and bioreactor devices are well known and exclusively directed to very small scale production volumes, such as in home brewing and for laboratory work. These bioreactor devices generally comprise a disposable bag which is typically cut open in order to harvest the cell/tissue yield, thus destroying any further usefulness of the bag. One such known disposable bioreactor is produced by Osmotec, Israel, (Agritech Israel, issue No. 11, Fall 1997, page 19) for small-scale use such as in laboratory research. This bioreactor comprises a conical bag having an inlet through which culture medium, air, inoculant and other optional additives may be introduced, and has a volume of only about 1.5 litres. Aeration is performed by introducing very small air bubbles which in many cases results in damage to cells, particularly in the case of plant cell cultures. In particular, these bags are specifically designed for a single culture/harvest cycle only, and the bag contents are removed by cutting off the bottom of the bag. These bags are therefore not directed towards an economical solution to the question of providing industrial quantities of the materials to be extracted from the culture, as discussed above.

[0005] The disposability of these bioreactor devices does not generally present an economic disadvantage to the user, since even the low capital costs of these items is offset against ease of use, storage and other practical considerations. In fact, at the low production levels that these devices are directed, such is the economy of the devices that there is no motivation to increase the complexity of the device or its operation for the sake of enabling such a device to be used continuously for more than one culturing/harvesting cycle.

[0006] Further, sterile conditions outside the disposable bioreactor devices are neither needed nor possible in many cases, and thus once opened to extract the harvestable yield, it is neither cost-effective, practical nor often possible to maintain the opening sterile, leading to contamination of the bag and whatever contents may remain inside. Thus, these disposable devices have no further use after one culturing cycle.

[0007] Disposable bioreactor devices are thus relatively inexpensive for the quantities and production volumes which are typically required by non-industrial-scale users, and are relatively easy to use by non-professional personnel. In fact it is this aspect of simplicity of use and low economic cost, which is related to the

low production volumes of the disposable devices, that is a major attraction of disposable bioreactor devices. Thus, the prior art disposable bioreactor devices have very little in common with industrial scale bioreacters - structurally, operationally or in the economics of scale - and in fact teach away from providing a solution to the problems associated with industrial scale bioreactors, rather than in any way disclose or suggest such a solution.

[0008] EP 0,200,792-A describes a sterilisable fermentor comprising a container formed by two rigid face plates and a length of foil hose clamped taut between the plates to provide an internal reaction space. The fitments are fixed to one of the face plates, and the fermentor can be inserted in a metallic cylinder during an in situ sterilisation. While the foil hose connecting the end plates may be disposable, the face plates are not. US 4,668,632 relates to a sparger useful for introducing gas into a liquid, and is composed of one or more gas-permeable members positioned between a gas inlet means and a gas distribution means. It also relates to a method and apparatus for culturing cells, wherein circulation of the apparatus is achieved by gas introduced into the medium via such a sparger. The major components are held together in a removable manner by means of screws. Further, while an outlet means is provided, this is for the liquid medium and the inlet means and outlet means of this reference merely enable the medium to flow into and out of the tube.

[0009] The present invention therefore represents a revolutionary solution to the aforementioned problems, providing a disposable bioreactor device for the large scale production of cell/tissue cultures. The device of the present invention, while disposable, is characterised in comprising a reusable harvesting outlet for enabling harvesting of at least a portion of the medium containing cells and/or tissue when desired, thereby enabling the device to be used continuously for 'one or more subsequent consecutive culturing/harvesting cycles. In an industrial environment, sterility of the harvesting outlet during and after harvesting may be assured to a significantly high degree at relatively low cost, by providing, for example, a sterile hood in which all the necessary connections and disconnections of services to and from the device may be performed. When eventually the device does become contaminated it may then be disposed of. Such devices may be cheaply manufactured, even for production volumes of 50 litres or more of culture. Further, the ability to perform a number of culturing/harvesting cycles is economically lucrative, lowering even further the effective cost per device. A battery of such devices can be economically arranged, and the number of devices in the battery may be controlled to closely match production to demand. Thus, the transition from pilot plant bioreactors to large scale production may also be achieved in a relatively simple and economic manner by adding more devices to the battery. Further, the relatively low production volume of each device, coupled with the lack of solid mixers, results in relatively higher yields as compared to typical stainless steel bioreactors.

[0010] An aim of the present invention is to provide a device, and associated method, for axenically culturing and harvesting cells and/or tissue, and which does not have the aforegoing disadvantages.

[0011] Another aim of the present invention is to provide such a device which is economical to produce and simple to use.

[0012] Another aim of the present invention is to provide such a device which is disposable, but nevertheless may be used continuously for a plurality of consecutive cycles of culturing and harvesting desired cells and/or tissues.

[0013] Another aim of the present invention is to provide such a device wherein inoculant is only required to be provided for the first culturing cycle, while inoculant for subsequent cycles is provided by a portion of the culture broth which remains in the device after harvesting same in a preceding cycle.

[0014] Another aim of the present invention is to provide a battery of such devices for industrial scale production of cells and/or tissues.

Summary of the Invention

[0015] A disposable device, and corresponding method according to claims 1 and 34 respectively, is provided. The said device may be disposed of when contaminated. In a second aspect of the invention, a battery of these devices, suitably interconnected, enables the scale of production of cells/tissues to be adjusted as required.

Description of the Figures

[0016]

Figures 1a and 1b illustrate the main components of a preferred embodiment of the present invention in front elevation and in cross-sectional side view, respectively.

Figures 2a and 2b illustrate the main components of a second embodiment of the present invention in front elevation and in cross-sectional side view, respectively.

Figure 3 illustrates the main components of a third embodiment of the present invention in cross-sectional side view.

Figure 4 illustrates the seam lines of the preferred embodiment of the present invention in front elevation.

Figure 5 illustrates the main components of a pre-

ferred embodiment of the battery of the present invention.

Description

[0017] The present invention relates to a disposable device for axenically culturing and harvesting cells and/or tissue in at least one cycle, said device comprising a sterilisable transparent and/or translucent disposable non-rigid container having a top end and a bottom end, which container may be at least partially filled with a suitable sterile biological cell and/or tissue culture medium and/or axenic inoculant and/or sterile air and/or required other sterile additives, said container comprising:-

(i) gas outlet means for removing excess air and/or waste gases from said container;

(ii) additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said container;
and characterised in further comprising

(iii) reusable harvesting means comprising suitable flow control means for enabling harvesting of at least a desired portion of the said medium containing cells and/or tissues when desired, thereby enabling said device to be used continuously for at least one further consecutive culturing/harvesting cycle,

wherein said container is adapted for enabling a remainder of said medium containing cells and/or tissue to remain in said container and to serve as inoculant for a next culture and harvest cycle.

[0018] The present invention further relates to such a device further comprising air inlet means for introducing sterile air in the form of bubbles into said culture medium through a first inlet opening, said air inlet means being connectable to a suitable air supply.

[0019] Thus, with reference to Figures 1, 2, and 3, corresponding respectively to a preferred, second and third embodiments of the device, the device, generally designated (10), comprises a transparent and/or translucent container (20), having a top end (26) and a bottom end (28). The said container (20) comprises a side wall (22) which is preferably substantially cylindrical. though other shapes such as rectangular or polyhedral, for example, may also be suitable. Preferably, the said bottom end (28) is suitably shaped to minimise sedimentation threat. For example, in the preferred embodiment, the said bottom end (28) is substantially frustro-conical or at least comprises upwardly sloping walls. In the second embodiment, the bottom end (28) comprises one upwardly sloping wall (29). In the third embodiment, the bottom end (28) is substantially cylindrical or alternatively convex. The aforementioned configurations of the bottom end (28), in conjunction with the location of the outlet (76) (hereinafter described) near the bottom end (28), enables air supplied via said outlet (76) to induce a mixing motion to the container contents at the bottom end (28) which effectively minimises sedimentation thereat. Nevertheless, the bottom end may be substantially flat in other embodiments of the present invention. The container (20) comprises an internal fillable volume (30) which is typically between 5 and 50 litres. though said device (10) may alternatively have an internal volume greater than 50 litres or less than 5 litres. Said internal volume (30) may be filled with a suitable sterile biological cell and/or tissue culture medium (65) and/or axenic inoculant (60) and/or sterile air and/or required other sterile additives such as antibiotics or fungicides for example, as hereinafter described. In the aforementioned embodiments, the container (20) is substantially non-rigid, being made preferably from a non-rigid plastics material chosen from the group comprising polyethylene, polycarbonate, a copolymer of polyethylene and nylon, PVC and EVA, for example. Optionally, the container (20) may be made from a laminate of more than one layer of said materials.

[0020] As shown for the third embodiment in Figure 3, the said container (20) may optionally comprise two concentric outer walls (24) to enhance mechanical strength and to minimise risk of contamination of the contents via the container walls.

[0021] In the preferred, second and third embodiments, said device (10) is for aerobic use. Thus the container (20) further comprises air inlet means for introducing sterile air in the form of bubbles (70) into said culture medium (65) through an air inlet opening (72). In the aforementioned embodiments, said air inlet means comprises a pipe (74) connectable to a suitable air supply (not shown) and extending from said inlet opening (72) to a location inside said container (20) at a distance d1 from the bottom of said bottom end (28), wherein d1 may be typically around 1 cm, though it could be greater or smaller than 1 cm. The said pipe (74) may be made from silicon or other suitable plastic material and is preferably flexible. The pipe (74) thus comprises an air outlet (76) of suitable diameter to produce air bubbles (70) of a required mean diameter. These bubbles not only aerate the medium (65), but also serve to mix the contents of the container, thereby minimising sedimentation at the bottom end (28) as well, as hereinbefore described. The size of the bubbles delivered by the air inlet means will vary according to the use of the device, ranging from well under 1 mm to over 10 mm in diameter.

[0022] In some cases, particularly relating to plant cells, small bubbles may actually damage the cell walls, and a mean bubble diameter of not less than 4 mm substantially overcomes this potential problem. In other cases, much smaller bubbles are beneficial, and a sparger may be used at the air outlet (76) to reduce the size of the bubbles. In yet other cases air bubbles of diameter 10 mm or even greater may be optimal. Optionally, said outlet (76) may be restrained in position at

said bottom end (28) by means of a tether (not shown) or other means known in the art.

[0023] In other embodiments, said device (10) is for anaerobic use, and thus does not comprise the said air inlet means.

[0024] The said container (20) further comprises gas outlet means for removing excess air and/or waste gases from said container (20). These gases collect at the said top end (26) of the said container (20). The said gas outlet means may comprise a pipe (90) having an inlet (96) at or near the said top end (26), at a distance d4 from the bottom of the said bottom end (28), wherein d4 is typically 90 cm for the preferred embodiment. The said pipe (90) may be made from silicon or other suitable plastic material and is preferably flexible. Said pipe (90) is connectable to a suitable exhaust means (not shown) by known means. The said exhaust means further comprises means, such as a suitable one-way valve or filter, for example, for substantially preventing introduction of contaminants into said container via said gas outlet means. At least a portion of the top end (26) may be suitably configured to facilitate the collection of waste gases prior to being removed via said inlet (96). Thus, in the preferred and second embodiments, the upper portion of the top end (26) progressively narrows to a minimum cross sectional area near the location of the inlet (96). Alternatively, at least the upper portion of the top end (26) may be correspondingly substantially frustro-conical or convex.

[0025] The said container (20) further comprises additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said container. In the aforementioned embodiments, said additive inlet means comprises a suitable pipe (80) having an outlet (86) preferably at or near the said top end (26), at a distance d3 from the bottom of the said bottom end (28), wherein d3 for the preferred embodiment is typically approximately 68 cm. The said pipe (80) may be made from silicon or other suitable plastic material and is preferably flexible. Said pipe (80) is connectable by known means to a suitable sterilised supply of said inoculant and/or said culture medium and/or said additives. Said additive inlet means further comprises means for substantially preventing introduction of contaminants into said container via said additive inlet means, and comprises, in these embodiments, a suitable one-way valve or filter (84). Typically, the level of contents of the container (20) remains below the level of the said outlet (86).

[0026] The said container (20) further comprises reusable harvesting means for harvesting at least a desired first portion of the said medium containing cells and/or tissue when desired, thereby enabling said device to be used continuously for at least one subsequent culturing cycle. A remaining second portion of said medium containing cells and/or tissue serves as inoculant for a next culture and harvest cycle, wherein said culture medium and/or said required additives provided. Said harvesting means may also be used to introduce the original volume of inoculant into the container, as well as for enabling the harvested material to flow therethrough and out of the container. In the aforementioned embodiments, said harvesting means comprises a pipe (50) having an inlet (52) in communication with said internal volume (30), and an outlet (56) outside said container (20). The said pipe (50) may be made from silicon or other suitable plastic material and is preferably flexible. Said pipe (50) is of a relatively large diameter, typically about 2 cm, since the harvested cell and/or tissue flow therethrough may contain clumps of cell particles that may clog narrower pipes. Preferably, said inlet (52) is located near the bottom end (28) of the said container (20), so that only the container contents above said inlet (52) are harvested. Thus, at the end of each harvesting cycle, said second portion of medium containing cells and/or tissues automatically remains at the said bottom end (28) of the said container (20), up to a level below the level (51) of the said inlet (52), which is at a distance d2 from the bottom of said bottom end (28). Typically, d2 is about 25 cm for the preferred embodiment. Alternatively, said inlet (52) may be located at the lowest point in the said container (20), wherein the operator would manually ensure that a suitable portion of medium containing cells and/or tissue would remain in the container (20) after harvesting a desired portion of medium and cells and/or tissue. Said harvest means further comprises flow control means such as a suitable valve (54) and/or an aseptic connector (55) for closing off and for permitting the flow of material into or out of said container (20) via said harvest means. Typically, said aseptic connector (55) is made from stainless steel, and many examples thereof are known in the art. Preferably, the said harvest means further comprise contamination prevention means for substantially preventing introduction of contaminants into said container via said harvesting means after harvesting. In the preferred, second and third embodiments, said contamination prevention means comprises a fluid trap (300). Said fluid trap (300) is preferably in the form of a substantially U-shaped hollow tube, one arm of which is mounted to the outlet (56) of the said harvesting means, and the other arm having an external opening (58). Harvested cells/tissue may flow out of the device (10) via said harvesting means, fluid trap (300) and said opening (58), to be collected thereafter in a suitable receiving tank as hereinafter described. After harvesting is terminated, air could possibly be introduced into the harvesting means via opening (56), accompanied by some back-flow of harvested material, thereby potentially introducing contaminants into the device. The said U-tube (300) substantially overcomes this potential problem by trapping some harvested material, i.e., cells/tissues, downstream of the opening (56) thereby preventing air, and possible contaminants, from entering the harvesting means. Once the harvesting means is closed off via said valve (54), the U-tube (300) is removed and typically sterilised for the

next use or discarded. The said U-tube (300) may be made from stainless steel or other suitable rigid plastic materials.

[0027] In the aforementioned embodiments, said remaining second portion of said medium containing cells and/or tissue typically comprises between 10% and 20% of the original volume of said culture medium and said inoculant, though said second portion may be greater than 20%, up to 45% or more, or less than 10%, down to 2.5% or less, of the said original volume, if required.

[0028] Said device (10) optionally further comprises attachment means for attaching same to an overhanging support structure. In the aforementioned embodiments, said support structure may comprise a bar (100) (Figures 1, 2) or rings (not shown). In the third embodiment, said attachment means may comprise a hook (25) preferably integrally attached to the said top end (26) of the said container (20). Alternatively, and as shown for the preferred and second embodiments in Figures 1 and 2 respectively, said attachment means may comprise a preferably flexible and substantially cylindrical loop (27) of suitable material, typically the same material as is used for the container (20), either integral with or suitably attached (via fusion welding, for example) to the top end (26) of the device.

[0029] The said container (20) may be formed by fusion bonding two suitable sheets of suitable material, as hereinbefore exampled, along predetermined seams. Referring to Figure 4, two sheets (200) of material may be cut in an approximately elongated rectangular shape and juxtaposed one over the other. The sheets are then fusion bonded together in a manner well known in the art to form seams along the peripheries (205) and (206) of the two longer sides, and along the periphery of one of the shorter ends (210), and again parallel and inwardly displaced thereto to form a seam (220) at the upper end of the container (20). The fusion weld seams (207) and (208) along the long sides and situated between these parallel short end seams (210) and (220) may be cut off or otherwise removed, effectively leaving a loop of material (27). The bottom end (28) of the container (20) is formed by fusion bonding the remaining short end of the sheets along two sloping seam lines, (230) and (240), mutually converging from the seams (205) and (206) of the long sides. Optionally, the two sloping seam lines (230) and (240) may be joined above the apex by another fusion welded seam line (260) approximately orthogonal to the long side seams (205) and (206). Prior to fusion welding the two sheets together, rigid plastic bosses (270), (290), (280) and (250) may be fusion welded at locations corresponding to the said air inlet means, gas outlet means, additive inlet means and harvesting means, respectively. These bosses provide suitable mechanical attachment points for each of the corresponding input and output means.

[0030] In all embodiments, the device (10) is made from a material or materials that are biologically compatible and which enable the container to be sterilised prior to first use.

[0031] The present invention also relates to a battery of disposable devices for axenically culturing and harvesting cells and/or tissue in cycles, wherein each of a plurality of these devices is structurally and operationally similar to said device (10), hereinbefore defined and described.

[0032] Referring to Figure 5, a battery (500) comprises a plurality of said devices (10) which are held on a frame or frames (not shown) by means of said attachment means. Typically, the battery may be divided into a number of groups, each group comprising a number of devices (10).

[0033] In the preferred embodiment, the said air inlet means of the devices (10) in each group are interconnected. Thus the said air inlet pipes (74) of each device (10) of the group is connected to common piping (174) having a free end (170), which is provided with an aseptic connector (175). Sterilised air is provided by a suitable air compressor (100) having a suitable sterilising means (110) such as one or more filters. The compressor (100) comprises a delivery pipe (101) having an aseptic connector (176) at its free end which is typically connectable to the said aseptic connector (175) located at the free end of common piping (174). This connection is made at the beginning of each run of growth/harvesting cycles in a mobile sterile hood (380) to ensure that sterile conditions are maintained during the connection. The sterile hood (380) provides a simple relatively low-cost system for connecting the various services, such as air, media, inoculant and harvested cells, to and from the group of devices (10) under substantially sterile conditions. Similarly, at the end of each run of growth/harvesting cycles, the connectors (175) and (176) are disconnected in the sterile hood (380), and the used devices are discarded, allowing the connector (175) at the compressor end to be connected to the connector (176) of a new group of devices. Sterilised air is typically provided continuously, or alternatively in predetermined pulses, during each culturing cycle.

[0034] In the preferred embodiment, excess air and/or waste gases from each of the said devices (10) is removed to the atmosphere via common piping (290) suitably connected to each corresponding gas outlet means (90). Said common piping (290) is provided with suitable means (210), such as one or more filters, for preventing contaminants from flowing into said devices (10). Alternatively, the gas outlet means (90) of each device (10) may be individually allowed to vent to the atmosphere, preferably via suitable filters which substantially prevent contaminants from flowing into the device (10).

[0035] Media and additives are contained in one or more holding tanks (340). For example, micro elements, macro elements and vitamins may be held in different tanks, while additives such as antibiotics and fungicides may also held in yet other separate tanks. Pumping

means (345) serving each tank enable the desired relative proportions of each component of the media and/or additives to be delivered at a predetermined and controllable flow rate to a static mixer (350), through which water - either distilled or suitably filtered and purified - flows from a suitable supply (360), preferably with the aid of a suitable pumping means (365) (Figure 5). By adjusting the flow rates of pumping means (345) and (365), for example, the concentration of media as well as additives available to be delivered into said devices (10) may be controlled. Media and/or additives mixed with water may then be delivered from the said static mixer (350) under sterile conditions via a filter (310) and a delivery pipe (370) having an aseptic connector (375) as its free end (390).

[0036] In the preferred embodiment, the inlet of additive pipe (80) of each corresponding device (10) in the group of said devices, are interconnected via common piping (180), which comprises at its free end a common aseptic connector (376). Said common aseptic connector (376) may then be connected, in the said sterile hood (380), to the aseptic connector (375) at the free end (390) of the media and additive pipe (370), thus enabling each device (10) of the battery, or of the group, to be supplied with media and additives. At the end of the life of the devices (10), and prior to discarding the same, the aseptic connectors (375) and (376) are disconnected in the sterile hood. The aseptic connector (375) is then ready to be connected to the new aseptic connector (376) of the next sterilised group of new devices (10) of the battery, ready for the next run of culturing/harvesting cycles.

[0037] The sterile hood (380) may also be utilised for connecting the media/additives tank (350) to each one of a number of groups of devices (10) in the battery, in turn, during the useful lives of the devices in these groups. Thus, when one group of devices has been serviced with media/additives, the aseptic connector (376) of this group is aseptically sealed temporarily in the sterile hood (380), which is then moved to the next group of devices where their common aseptic connector (376) is connected to the sterile connector (375) of the pipe (370), thus enabling this group of devices to be serviced with media/additives.

[0038] In another embodiment, said mobile sterile hood (380) may be used to connect together the free end (390) of a preferably flexible delivery pipe connected to said static mixing tank (350), to the additive inlet means of each device (10) in turn. The said sterile hood (380) may then be moved from one said device (10) to the next, each time the said end (390) being connected to the inlet end of the corresponding pipe (80) to enable media to be provided to each device in turn. The sterile hood (380), together with aseptic connecting means, preferably made from stainless steel, at said end (390) and the inlet of the pipe (80) of the corresponding device (10), respectively, enable each device (10) to be easily connected and subsequently disconnected to the end (390) and thus to the media supply, under sterile conditions. Many other examples of suitable connecting means for connecting two pipes together are well known in the art. Suitable filters are provided at the end (390) and at the pipe (80), respectively, to prevent or at least minimise potential contamination of the container contents. The sterile hood (380) may thus be automatically or manually moved from device (10) to device (10), and at each device in turn, an operator may connect the device (10) to the media supply using the sterile hood (380), fill the device with a suitable quantity of media and/or additives, and subsequently disconnect the sterile hood (380) from the device, to then move on to the next device. Of course, the end (390) may be adapted to comprise a plurality of connecting means (375) rather than just a single sterilised connecting means (375), so that rather than one, a similar plurality of devices (10) having corresponding connecting means (376) may be connected at a time to the media supply via the trolley (380).

[0039] Each time, prior to connecting said end (390) to each device or set or group of devices, the corresponding connecting means (375) and (376) are typically autoclave sterilised.

[0040] In yet another embodiment of the battery, a single pipe or a set of pipes (not shown) connect said static mixer (350), to a said device (10) or to a corresponding set of devices (10), respectively, at a time, wherein a conveyor system transports the device (10) or set of devices (10) to the said single pipe or set of pipes, respectively, or vice versa. After filling the said device (10) or set of devices (10), the conveyor enables a further device (10) or set of devices (10) to be connected to the static mixer (350) by means of the said single pipe or set of pipes, respectively.

[0041] In the preferred embodiment, the harvesting means of each of the devices (10) of the group are interconnected. Thus the harvesting pipes (50) of each said device (10) is connected to common harvesting piping (154) having a free end (150), which is provided with an aseptic connector (155). Preferably, each of the said harvesting pipes (50) may comprise a valve (54), as hereinbefore described, to close off or permit the flow of harvested cells from each corresponding device (10). Thus, for example, if it is determined that a number of devices in a particular group are contaminated, while the other devices are not, then the cells in these latter devices may be harvested without fear of contamination from the former devices, so long as the valves (54) of the contaminated devices remain closed. Preferably, said common piping further comprises a common shut-off valve (259) upstream of the said aseptic connector (155). Preferably, said contamination prevention means is provided for substantially preventing introduction of contaminants into said container via said harvesting means after harvesting. In the preferred embodiment, said contamination prevention means comprises a substantially U-shaped fluid trap (400), having an aseptic

connector (156) at one arm thereof, the other arm having an opening (158) in fluid communication with a receiving tank (590). The aseptic connectors (155) and (156) are then interconnected in the said mobile sterile hood (380) under sterile conditions. Harvesting is then effected by opening the valves (54) of all the devices in the group which are not contaminated, as well as common valve (259). Cells from the group will then flow into the receiving tank (590), preferably under gravity, though in some cases a suitable pump may be used. After harvesting is completed, the aseptic connectors (155) and (156) may be disconnected in the said sterile hood (380), which can then be moved to the next group of devices (10): the corresponding aseptic connector (155) of this group may then be interconnected with aseptic connector (156) of the U-tube (400), and thereby enable the cells of this group of devices to be harvested.

[0042] In another embodiment, a single pipe or a set of pipes (not shown) may connect said common receiving tank to a said device (10) or a corresponding set of devices (10), respectively, at a time, wherein a conveyor system transports the device (10) or set of devices (10) to the said single pipe or set of pipes, respectively, or vice versa. After harvesting the said device (10) or set of devices (10), the conveyor enables a further device (10) or set of devices (10) to be connected to the said common receiving tank by means of the said single pipe or set of pipes, respectively.

[0043] In another embodiment, each device (10) may be individually harvested, wherein the said harvesting means of each device comprises said contamination prevention means for substantially preventing introduction of contaminants into said container via said harvesting means after harvesting. In this embodiment, said contamination prevention means comprises said U-shaped fluid trap (400) as hereinbefore described, having an aseptic connector (156) at one arm thereof, the other arm having an opening (158) in fluid communication with a receiving tank (590). The said harvesting means comprises an aseptic connector (55) which may be connected to the aseptic connector (156) of the fluid trap (400) in the said mobile sterile hood (380) under sterile conditions. Harvesting is then effected by opening the valve (54) of the device, wherein cells will then flow into the receiving tank, preferably under gravity, though in some cases a suitable pump may be used. After harvesting is completed, these aseptic connectors, (55) and (156), may be disconnected in the said sterile hood (380), which can then be moved to the next device (10): the corresponding aseptic connector (55) of the harvesting means of this device may then be interconnected with aseptic connector (156) of the U-tube (400), and thereby enable the cells of this next device to be harvested.

[0044] In the preferred embodiment, said harvesting means may also be used for initially providing inoculant at the start of a new run of growth/harvesting cycles. Thus, inoculant may be mixed with sterilised medium in a suitable tank having a delivery pipe comprising at its free end an aseptic connector which is connected to the said aseptic connector (155) of the common harvesting piping (154) in the said sterile hood (380). Inoculant may then be allowed to flow under gravity, or with the aid of a suitable pump, to each of the devices (10) of the group via said common harvesting piping (154), after which the aseptic connectors are disconnected in the sterile hood.

[0045] Alternatively, the said inoculant may be introduced into the devices via the said additive inlet means, in particular the said additive means common piping (180), in a similar manner to that hereinbefore described regarding the harvesting means and the common harvesting piping (155), *mutatis mutandis*.

[0046] The present invention also relates to a method for culturing and harvesting cells and/or tissue in a multiple-use disposable device comprising the steps of:-

a) providing said device (10), hereinbefore defined ;
b) providing sterile air to said container via said air inlet means during each cycle, either continuously or in pulses:
c) providing sterile said culture medium and/or sterile said additives via said additive inlet means;
d) providing axenic inoculant via said harvesting means;
e) optionally illuminating said container with external light means;
f) allowing said cells and/or tissue to grow in said medium to a desired yield;
g) continuously allowing excess air and/or waste gases to leave said container via said gas outlet means;
h) checking for contaminants and/or the quality of the cells/tissues which are produced in said container: if contaminants are found to be present or the cells/tissues which are produced are of poor quality, the device and its contents are disposed of; if contaminants are not found. step i) is executed;
i) harvesting at least said desired first portion of the said medium containing cells and/or tissue, while leaving a remaining said second portion of medium containing cells and/or tissue in said container, wherein said second portion of medium may serve as inoculant for a next culture/harvest cycle;
j) providing sterile said culture medium and/or sterile said additives for the next culture/harvest cycle via said additive inlet means;
k) repeating steps b), e), f), g), h), i) and j) a plurality of times until in h) the said contaminants are found to be present or the cells/tissues which are produced are of poor quality, whereupon the device and its contents are disposed of.

[0047] The present invention also relates to a method for axenically culturing and harvesting cells and/or tissue anerobically in a battery of disposable devices comprising the steps of :-

a) providing a battery (500) of at least one group of said devices (10), wherein said devices do not comprise air inlet means, and for at least one said device (10) thereof:
b) providing axenic inoculant to said device via said common harvesting piping;
c) providing sterile said culture medium and/or sterile said additives to said device via said common additive inlet piping;
d) optionally illuminating said device with external light means;
e) allowing said cells and/or tissue in said device to grow in said medium to a desired yield;
f) allowing excess air and/or waste gases to leave said device continuously via said common gas outlet piping;
g) checking for contaminants and/or the quality of the cells/tissues which are produced in said device: if in the said device contaminants are found or the cells/tissues which are produced are of poor quality, the said harvesting means of said device is closed off preventing contamination of other said devices of said battery; if in all of the said devices of the said battery contaminants are found or the cells/tissues which are produced therein are of poor quality, all the devices and their contents are disposed of: if contaminants are not found and the quality of the produced cells/tissues is acceptable, the device is considered harvestable and step h) is executed;
h) for each said harvestable device of step g), harvesting at least said desired first portion of the said medium containing cells and/or tissue via said common harvesting piping and said contamination prevention means to a suitable receiving tank, while leaving said second portion of medium containing cells and/or tissue in said container, wherein said second portion of medium serves as inoculant for a next culture/harvest cycle;
i) providing sterile said culture medium and/or sterile said additives for the next culture/harvest cycle via said additive inlet means;
j) repeating steps d), e), f), g), h) and i) a plurality of times until in g) the said contaminants are found or the cells/tissues which are produced are of poor quality for all of the said devices of the said battery, whereupon the said contamination prevention means are disconnected from the said common harvesting means and the said devices and their contents are disposed of.

[0048] The present invention also relates to a method for axenically culturing and harvesting cells and/or tissue aerobically in a battery of disposable devices comprising the steps of :-

a) providing a battery (500) of at least one group of said devices (10), wherein said devices comprise air inlet means as hereinbefore described, and for at least one said device (10) thereof:
b) providing axenic inoculant to said device via said common harvesting piping;
c) providing sterile said culture medium and/or sterile said additives to said device via said common additive inlet piping;
d) providing sterile air to said device via said common air inlet piping;
e) optionally illuminating said device with external light means;
f) allowing said cells and/or tissue in said device to grow in said medium to a desired yield;
g) allowing excess air and/or waste gases to leave said device continuously via said common gas outlet piping;
h) checking for contaminants and/or the quality of the cells/tissues which are produced in said device: if in the said device contaminants are found or the cells/tissues which are produced are of poor quality, the said harvesting means of said device is closed off preventing contamination of other said devices of said battery; if in all the said devices of the said battery contaminants are found or the cells/tissues which are produced therein are of poor quality, all the devices and their contents are disposed of; if contaminants are not found and the quality of the produced cells/tissues is acceptable, the device is considered harvestable and step i) is executed;
i) for each said harvestable device of step h), harvesting at least said desired first portion of the said medium containing cells and/or tissue via said common harvesting piping and said contamination prevention means to a suitable receiving tank, while leaving said second portion of medium containing cells and/or tissue in said container, wherein said second portion of medium serves as inoculant for a next culture/harvest cycle;
j) providing sterile said culture medium and/or sterile said additives for the next culture/harvest cycle via said additive inlet means;
k) repeating steps d), e), f), g), h), i) and j) a plurality of times until in h) the said contaminants are found or the cells/tissues which are produced are of poor quality for all of the said devices of the said battery, whereupon the said contamination prevention means are disconnected from the said common harvesting means and the said devices and their contents are disposed of.

[0049] Typically, a water purification system supplies deionised and pyrogen free water to a tank comprising concentrated media, and diluted media is then pumped to the said device (10) via said additive inlet means. Filters, typically 0.2μm, are installed in the feed pipes and also just upstream of the said additive inlet means to minimise risk of contamination of the container contents in each device (10). Alternatively or additionally, a one-way valve may be also be used to minimise this risk.

[0050] For the first culturing cycle of each device (10), inoculant, typically a sample of the type of cell that it is required to harvest in the said device (10), is pre-mixed with media or water in a steam sterilised container and is introduced into the device (10) via the harvesting means. Media is then introduced into the device (10) via said additive input means. For subsequent cycles, only media and/or additives are introduced, as hereinbefore described.

[0051] Typically, an air compressor provides substantially sterilised air to each said device (10), via a number of filters: a coarse filter for removing particles, a dryer and humidity filter for removing humidity, and a fine filter, typically 0.2μm, for removing contaminants. Preferably, another filter just upstream of the said air inlet means further minimises the risk of contamination of the container contents.

[0052] For each said device (10), all connections to the container (20), i.e., to said air inlet means, to said additive inlet means, and preferably also to the gas outlet means and to the harvesting means are autoclave sterilised prior to use, and sterility is maintained during connection to peripheral equipment, including, for example, said air supply and said exhaust means by performing the connections in the sterile hood as hereinbefore described.

[0053] Temperature control for each device (10) is preferably provided by suitable air conditioning means. Optional illumination of the device may be provide by suitable fluorescent light means suitably arranged around the said device (10), when required for cell growth.

[0054] During each culturing cycle of each device (10), the contents of each corresponding container (20) are typically aerated and mixed for about 7 to about 14 days, or longer, under controlled temperature and lighting conditions.

[0055] At the end of the culturing cycle for each device (10), the corresponding said harvesting means is typically connected to a presterilised environment by means of suitable connectors which are sterilised prior and during connection, as hereinbefore described. Harvesting is then effected, leaving behind between about 2.5% to about 45%, though typically between about 10% to about 20%, of cells and/or tissue to serve as inoculant for the next cycle.

[0056] The harvested cells/tissues may then be dried, or extracted, as required.

[0057] The present invention will be described in more detail with reference to the following example, which is not intended to limit the scope of the invention.

Example Culturing Vinca Cells

[0058] A group of 10 bioreactors (each a device according to the invention), each with a container made from polyethylene-nylon copolymer, (0.1 mm wall thickness, 20 cm diameter, 1.2 m height), complete with 30 mm ports at 5 cm (for air inlet means), 25 cm (for harvesting means), 68 cm (additive inlet means), and 90 cm (gas outlet means) from the bottom, effective fillable volume about 10 liters was used. The bioreactors, together with their fittings, were sterilized by gamma irradiation (2.5 mRad).

[0059] Nine liters of Schenk & Hildebrandt mineral/vitamin medium, 2 mg/l each of chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid, 0.2 mg/l kinetin, 3% sucrose, and 900 ml packed volume initial inoculum of line V24 *Catharanthus roseus* (Vinca) cells were introduced into each bioreactor. The volume of air above the surface of the medium was 3 1. Aeration was carried out using a flow volume of 1.5 l/min sterile air, provided through a 4 mm orifice (air inlet means), located 1 cm from the bottom of the container.

[0060] The bioreactors were mounted in a controlled temperature room (25°C) and culturing was continued for 10 days, until the packed volume increased to about 7.5 1 (75% of the total volume; a doubling rate of 2 days during the logarithmic phase). At this time point, cells were harvested by withdrawing 9 liters of medium and cells through the harvesting means and 9 liters of fresh sterile medium together with the same additives were added via the additive inlet means. Cells were again harvested as above at 10-day intervals, for 6 additional cycles, at which time the run was completed.

[0061] A total weight of 6.5 kg fresh cells (0.5 kg dry weight) was thus collected over seven 10-day periods of time, from each of the 10 1 capacity bioreactors. These cells had a 0.6% content of total alkaloids, the same as the starting line.

[0062] Although only a few embodiments have been described in detail in the foregoing description, the present invention is not limited thereto and is only defined by the scope of the claims.

Claims

1. A disposable device (10) for axenically culturing and harvesting cells and/or tissue in at least one cycle, said device comprising a sterilisable transparent and/or translucent disposable non-rigid container (20) having a top end (26) and a bottom end (28), which container may be at least partially filled with a suitable sterile biological cell and/or tissue culture medium and/or axenic inoculant and/or sterile air and/or required other sterile additives, said container comprising:-

   (i) gas outlet means (90, 96) for removing excess air and/or waste gases from said container;

   (ii) additive inlet means (80. 86) for introducing said inoculant and/or said culture medium and/or said additives into said container;

and **characterised in** further comprising

(iii) reusable harvesting means (50, 52, 56) comprising suitable flow control means (54, 55) for enabling harvesting of at least a desired portion of the said medium containing cells and/or tissues when desired, thereby enabling said device to be used continuously for at least one further consecutive culturing/harvesting cycle,

wherein said container is adapted (d2) for enabling a remainder of said medium containing cells and/or tissue to remain in said container to serve as inoculant for a next culture and harvesting cycle.

2. The device of claim 1, further comprising air inlet means for introducing sterile air in the form of bubbles into said culture medium through a first inlet opening, wherein said air inlet means is connectable to a suitable air supply.

3. The device of claim 1 or claim 2, said harvesting means comprising contamination prevention means for substantially preventing introduction of contaminants into said container via said harvesting means.

4. The device as claimed in any preceding claim, wherein said container is made from a non-rigid plastics material.

5. The device of claim 4, wherein said material is selected from the group comprising polyethylene, polycarbonate, a copolymer of polyethylene and nylon, PVC and EVA.

6. The device as claimed in claims 4 or 5, wherein said container is made from a laminate of more than one layer of said materials.

7. The device as claimed in any one of claims 4 to 6, wherein said container is formed by fusion bonding two suitable sheets of said material along predetermined seams.

8. The device as claimed in any preceding claim, wherein said air inlet means comprises an air inlet pipe extending from said inlet opening to a location inside said container at or near the said bottom end thereof.

9. The device as claimed in any preceding claim, wherein at least a some of said air bubbles comprise a mean diameter of between about 1 mm and about 10 mm.

10. The device as claimed in any preceding claim, wherein at least some of said air bubbles comprise a mean diameter of about 4 mm.

11. The device as claimed in any preceding claim, wherein said container comprises a suitable filter mounted on said gas outlet means for substantially preventing introduction of contaminants into said container via said gas outlet means.

12. The device as claimed in any preceding claim, wherein said container further comprises a suitable filter mounted on said additive inlet means for substantially preventing introduction of contaminants into said container via said additive inlet means.

13. The device as claimed in any preceding claim, wherein said contamination prevention means comprises a U-shaped fluid trap, wherein one arm thereof is aseptically mounted to an external outlet of said harvesting means by suitable aseptic connection means.

14. The device as claimed in any preceding claim, wherein said harvesting means is located at the bottom of said bottom end of said container.

15. The device as claimed in any one of claims 1 to 13, wherein said harvesting means is located near the bottom of said bottom end of said container, such that at the end of each harvesting cycle said remainder of said medium containing cells and/or tissue automatically remains at the said bottom end of said container up to a level below the level of said harvesting means.

16. The device as claimed in any preceding claim, wherein said remainder of said medium containing cells and/or tissue comprises between about 2.5% and about 45%, and preferably between about 10% and about 20% of the original volume of said culture medium and said inoculant.

17. The device as claimed in any preceding claim, wherein said bottom end is substantially convex.

18. The device as claimed in any one of claims 1 to 16, wherein said bottom end is substantially frusta-conical.

19. The device as claimed in any preceding claim, wherein said container comprises an internal fillable volume of between about 5 litres and about 50 litres.

20. The device as claimed in any preceding claim, wherein said device further comprises suitable attachment means for attaching same to a suitable support structure.

21. The device as claimed in claim 20, wherein said at-

tachment means comprises a loop of material preferably integrally attached to said top end of said container.

22. A battery of said devices, comprising at least two said disposable devices as claimed in any preceding claim.

23. The battery of claim 22, wherein said devices are supported by a suitable support structure via the said attachment means of each said device.

24. The battery of claims 22 or 23, wherein the said gas outlet means of each said device is suitably connected to a common gas outlet piping which optionally comprises suitable means for preventing contaminants from flowing into said devices.

25. The battery of claim 24, wherein said means for preventing contaminants from flowing into said devices comprises a suitable filter.

26. The battery of any one of claims 22 to 25, wherein the said additive inlet means of each said device is suitably connected to a common additive inlet piping having a free end optionally comprising suitable aseptic connecting means thereat.

27. The battery of claim 26, wherein said free end is connectable to a suitable supply of medium and/or additives.

28. The battery of any one of claims 22 to 27, wherein the said harvesting means of each said device is suitably connected to a common harvesting piping having a free end optionally comprising suitable aseptic connecting means thereat.

29. The battery of claim 28, further comprising contamination prevention means for substantially preventing introduction of contaminants into said container via said common harvesting piping.

30. The battery of claim 29, wherein said contamination prevention means comprises a U-shaped fluid trap, wherein one arm thereof is free having an opening and wherein the other end thereof is aseptically mountable to said free end of said common harvesting piping via suitable aseptic connection means.

31. The battery of claims 30, wherein the said free end of said U-tube is connectable to a suitable receiving tank.

32. The battery of any one of claims 22 to 31, wherein the said air inlet means of each said device is suitably connected to a common air inlet piping having a free end optionally comprising suitable aseptic connecting means thereat.

33. The battery of claim 32, wherein said free end is connectable to a suitable air supply.

34. A method for axenically culturing and harvesting cells and/or tissue in a disposable device comprising the steps of :-

a) providing said device which comprises a sterilisable transparent and/or translucent disposable non-rigid container having a top end and a bottom end, which container may be at least partially filled with a suitable sterile biological cell and/or tissue culture medium and/or axenic inoculant and/or sterile air and/or other sterile required additives, said container comprising:-

(i) gas outlet means for removing excess air and/or waste gases from said container;
(ii) additive inlet means for introducing said inoculant and/or said culture medium and/or said additives into said container;
and **characterised in** further comprising:
(iii) reusable harvesting means comprising suitable flow control means for enabling harvesting of at least a desired portion of the said medium containing cells and/or tissue when desired, thereby enabling said device to be used continuously for at least one further consecutive culturing/harvesting cycle, wherein said container is adapted for enabling a remainder of said medium containing cells and/or tissue to remain in said container to serve as inoculant for a next culture and harvesting cycle;

b) providing axenic inoculant via said harvesting means;
c) providing sterile said culture medium and/or sterile said additives via said additive inlet means;
d) optionally illuminating said container with external light means;
e) allowing said cells and/or tissue to grow in said medium to a desired yield;
f) allowing excess air and/or waste gases to leave said container continuously via said gas outlet means;
g) checking for contaminants and/or the quality of the cells/tissues which are produced in said container: if contaminants are found or the cells/tissues which are produced are of poor quality, the device and its contents are disposed of; if contaminants are not found, step h) is executed;
h) harvesting said desired portion of the said

medium containing cells and/or tissue, while leaving said remainder of medium containing cells and/or tissue in said container, wherein said remainder of medium serves as inoculant for a next culture/harvest cycle;
i) providing sterile said culture medium and/or sterile said additives for the next culture/harvest cycle via said additive inlet means;
j) repeating steps d), e), f), g), h) and i) a plurality of times until in g) the said contaminants are found or the cells/tissues which are produced are of poor quality, whereupon the device and its contents are disposed of.

35. The method of claim 34, wherein said device further comprises air inlet means for introducing sterile air in the form of bubbles into said culture medium through a first inlet opening connectable to a suitable sterile air supply, said method further comprising the step of providing sterile air to said air inlet means during the first and each subsequent cycle.

36. The method of claim 35, wherein said sterile air is supplied continuously throughout at least one culturing cycle.

37. The method of claim 35, wherein said sterile air is supplied in pulses during at least one culturing cycle.

38. A method for axenically culturing and harvesting cells and/or tissue in a battery of disposable devices comprising the steps of :-

a) providing a battery of devices as claimed in claim 30, and for at least one said device thereof:
b) providing axenic inoculant to said device via said common harvesting piping;
c) providing sterile said culture medium and/or sterile said additives to said device via said common additive inlet piping;
d) optionally illuminating said device with external light means;
e) allowing said cells and/or tissue in said device to grow in said medium to a desired yield;
f) allowing excess air and/or waste gases to leave said device continuously via said common gas outlet piping;
g) checking for contaminants and/or the quality of the cells/tissues which are produced in said device: if in the said device contaminants are found or the cells/tissues which are produced are of poor quality, the said harvesting means of said device is closed off preventing contamination of other said devices of said battery; if in all of the said devices of the said battery contaminants are found or the cells/tissues which are produced therein are of poor quality, all the devices and their contents are disposed of; if contaminants are not found and the quality of the produced cells/tissues is acceptable, the device is considered harvestable and step h) is executed;
h) for each said harvestable device of step g), harvesting said desired portion of the said medium containing cells and/or tissue via said common harvesting piping and said contamination prevention means to a suitable receiving tank, while leaving said remainder of medium containing cells and/or tissue in said container, wherein said second portion of medium serves as inoculant for a next culture/harvest cycle;
i) providing sterile said culture medium and/or sterile said additives for the next culture/harvest cycle via said additive inlet means;
j) repeating steps d), e), f), g), h) and i) a plurality of times until in g) the said contaminants are found or the cells/tissues which are produced are of poor quality for all of the said devices of the said battery, whereupon the said contamination prevention means are disconnected from the said common harvesting means and the said devices and their contents are disposed of.

39. A method for axenically culturing and harvesting cells and/or tissue in a battery of disposable devices comprising the steps of :-

a) providing a battery of devices as claimed in claim 33, and for at least one said device thereof:
b) providing axenic inoculant to said device via said common harvesting piping;
c) providing sterile said culture medium and/or sterile said additives to said device via said common additive inlet piping;
d) providing sterile air to said device via said common air inlet piping;
e) optionally illuminating said device with external light means;
f) allowing said cells and/or tissue in said device to grow in said medium to a desired yield;
g) allowing excess air and/or waste gases to leave said device continuously via said common gas outlet piping;
h) checking for contaminants and/or the quality of the cells/tissues which are produced in said device: if in the said device contaminants are found or the cells/tissues which are produced are of poor quality, the said harvesting means of said device is closed off preventing contamination of other said devices of said battery; if in all of the said devices of the said battery contaminants are found or the cells/tissues which

are produced therein are of poor quality, all the devices and their contents are disposed of; if contaminants are not found and the quality of the produced cells/tissues is acceptable, the device is considered harvestable and step i) is executed;
i) for each said harvestable device of step h), harvesting at least a desired portion of the said medium containing cells and/or tissue via said common harvesting piping and said contamination prevention means to a suitable receiving tank, while leaving said remainder of medium containing cells and/or tissue in said container, wherein said remainder of medium serves as inoculant for a next culture/harvest cycle;
j) providing sterile said culture medium and/or sterile said additives for the next culture/harvest cycle via said additive inlet means;
k) repeating steps d), e), t), g), h), i) and j) a plurality of times until in h) the said contaminants are found or the cells/tissues which are produced are of poor quality for all of the said devices of the said battery, whereupon the said contamination prevention means are disconnected from the said common harvesting means and the said devices and their contents are disposed of.

Patentansprüche

1. Einwegvorrichtung (10) für das axenische Kultivieren und Ernten von Zellen und/oder Gewebe in mindestens einem Zyklus, wobei die Vorrichtung einen sterilisierbaren transparenten und/oder lichtdurchlässigen nicht starren Einwegbehälter (20) mit einem oberen Ende (26) und einem unteren Ende (28) umfasst, welcher zumindest teilweise mit einem geeigneten sterilen biologischen Zell- und/oder Gewebekulturmedium und/oder axenischen Inokulant und/oder steriler Luft und/oder erforderlichen anderen sterilen Zusätzen gefüllt werden kann, wobei der Behälter Folgendes umfasst:

   (i) Gasauslassmittel (90, 96) für das Ablassen überschüssiger Luft und/oder Abgase aus dem Behälter;

   (ii) Zusatzeinlassmittel (80, 86) für das Einbringen des Inokulants und/oder des Kulturmediums und/oder der Zusätze in den Behälter;
   und **dadurch gekennzeichnet, dass** er weiterhin umfasst:

   (iii) wiederverwendbare Erntemittel (50, 52, 56), welche geeignete Stromsteuermittel (54, 55) für das Ermöglichen des Erntens mindestens eines gewünschten Teils des die Zellen und/oder das Gewebe enthaltenden Mediums, wenn dies gewünscht ist, umfassen, wodurch die Vorrichtung ständig für mindestens einen weiteren folgenden Kultivier-/Erntezyklus verwendet werden kann,

   wobei der Behälter so ausgelegt (d2) ist, dass ein Rest des die Zellen und/oder das Gewebe enthaltenden Mediums in dem Behälter verbleiben kann, um als Inokulant für einen nächsten Kultur- und Erntezyklus zu dienen.

2. Vorrichtung nach Anspruch 1, welche weiterhin Lufteinlassmittel für das Einbringen steriler Luft in Form von Bläschen in das Kulturmedium durch eine erste Einlassöffnung umfasst, wobei das Lufteinlassmittel mit einer geeigneten Luftzufuhr verbindbar ist.

3. Vorrichtung nach Anspruch 1 oder 2, bei der das Erntemittel ein Verunreinigungsverhinderungsmittel für das wesentliche Verhindern des Einbringens von Verunreinigungen über das Erntemittel in den Behälter umfasst.

4. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet dass** der Behälter aus einem nicht starren Kunststoffmaterial gefertigt ist.

5. Vorrichtung nach Anspruch 4, **dadurch gekennzeichnet, dass** das Material aus der Gruppe, welche Polyethylen, Polycarbonat, ein Copolymer von Polyethylen und Nylon, PVC und EVA umfasst, gewählt wird.

6. Vorrichtung nach Anspruch 4 oder 5, **dadurch gekennzeichnet, dass** der Behälter aus einem Laminat von mehr als einer Schicht dieser Materialien gefertigt ist.

7. Vorrichtung nach einem der Ansprüche 4 bis 6, **dadurch gekennzeichnet, dass** der Behälter durch Direktbonden von zwei geeigneten Platten dieses Materials entlang vorbestimmten Falzen gebildet wird.

8. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** das Lufteinlassmittel ein sich von der Einlassöffnung zu einem Ort in dem Behälter am oder nahe des unteren Endes desselben erstreckendes Lufteinlassrohr umfasst.

9. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** mindestens einige der Luftbläschen einen mittleren Durchmesser von etwa 1 mm bis etwa 10 mm aufweisen.

10. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** mindestens einige der Luftbläschen einen mittleren Durchmesser von etwa 4 mm aufweisen.

11. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** der Behälter einen an dem Gasauslassmittel angebrachten geeigneten Filter für das wesentliche Verhindern eines Einbringens von Verunreinigungen über das Gasauslassmittel in den Behälter umfasst.

12. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** der Behälter weiterhin einen an dem Zusatzeinlassmittel angebrachten geeigneten Filter für das wesentliche Verhindern eines Einbringens von Verunreinigungen über das Zusatzeinlassmittel in den Behälter umfasst.

13. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** das Verunreinigungsverhinderungsmittel eine U-förmige Fluidfalle umfasst, bei der ein Arm derselben an einem externen Auslass des Erntemittels durch geeignete aseptische Verbindungsmittel aseptisch angebracht ist.

14. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** das Erntemittel am Boden des unteren Endes des Behälters angeordnet ist.

15. Vorrichtung nach einem der Ansprüche 1 bis 13, **dadurch gekennzeichnet, dass** das Erntemittel nahe des Bodens des unteren Endes des Behälters angeordnet ist, so dass am Ende jedes Erntezyklus der Rest des die Zellen und/oder das Gewebe enthaltenden Mediums automatisch am unteren Ende des Behälters bis zu einem Füllstand unter der Höhe des Erntemittels verbleibt.

16. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** der Rest des die Zellen und/oder das Gewebe enthaltenden Mediums etwa 2,5 % bis etwa 45 % und vorzugsweise etwa 10 % bis etwa 20 % des ursprünglichen Volumens des Kulturmediums und des Inokulants umfasst.

17. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** das untere Ende im Wesentlichen konvex ist.

18. Vorrichtung nach einem der Ansprüche 1 bis 16, **dadurch gekennzeichnet, dass** das untere Ende im Wesentlichen stumpfkegelig ist.

19. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** der Behälter ein füllbares Innenvolumen von etwa 5 Liter bis etwa 50 Liter aufweist.

20. Vorrichtung nach einem der vorstehenden Ansprüche, **dadurch gekennzeichnet, dass** die Vorrichtung weiterhin geeignete Befestigungsmittel zum Befestigen derselben an einem geeigneten Halterungsaufbau umfasst.

21. Vorrichtung nach Anspruch 20, **dadurch gekennzeichnet, dass** das Befestigungsmittel eine Materialschlaufe umfasst, die vorzugsweise an dem oberen Ende des Behälters einstückig angebracht ist.

22. Gruppe besagter Vorrichtungen, welche mindestens zwei Einwegvorrichtungen nach einem der vorstehenden Ansprüche umfasst.

23. Gruppe nach Anspruch 22, **dadurch gekennzeichnet, dass** die Vorrichtungen durch einen geeigneten Halterungsaufbau über das Befestigungsmittel jeder Vorrichtung gehalten werden.

24. Gruppe nach Anspruch 22 oder 23, **dadurch gekennzeichnet, dass** das Gasauslassmittel jeder Vorrichtung mit einem gemeinsamen Gasauslassrohrsystem, welches optional geeignete Mittel zum Verhindern eines Strömens von Verunreinigungen in die Vorrichtungen umfasst, geeignet verbunden ist.

25. Gruppe nach Anspruch 24, **dadurch gekennzeichnet, dass** das Mittel zum Verhindern eines Strömens von Verunreinigungen in die Vorrichtungen einen geeigneten Filter umfasst.

26. Gruppe nach einem der Ansprüche 22 bis 25, **dadurch gekennzeichnet, dass** das Zusatzeinlassmittel jeder Vorrichtung mit einem gemeinsamen Zusatzeinlassrohrsystem mit einem freien Ende, welches daran geeignete aseptische Verbindungsmittel umfasst, geeignet verbunden ist.

27. Gruppe nach Anspruch 26, **dadurch gekennzeichnet, dass** das freie Ende mit einer geeigneten Zufuhr für Medium und/oder Zusätzen verbindbar ist.

28. Gruppe nach einem der Ansprüche 22 bis 27, **dadurch gekennzeichnet, dass** das Erntemittel jeder Vorrichtung mit einem gemeinsamen Ernterohrsystem mit einem freien Ende, welches daran optional geeignete aseptische Verbindungsmittel umfasst, geeignet verbunden ist.

29. Gruppe nach Anspruch 28, welche weiterhin Ver-

unreinigungsverhinderungsmittel für das wesentliche Verhindern eines Einbringens von Verunreinigungen über das gemeinsame Ernterohrsystem in den Behälter umfasst.

30. Gruppe nach Anspruch 29, **dadurch gekennzeichnet, dass** das Verunreinigungsverhinderungsmittel eine U-förmige Fluidfalle umfasst, bei der ein Arm derselben frei ist und eine Öffnung aufweist, und dass das andere Ende derselben an dem freien Ende des gemeinsamen Ernterohrsystems durch geeignete aseptische Verbindungsmittel aseptisch anbringbar ist.

31. Gruppe nach Anspruch 30, **dadurch gekennzeichnet, dass** das freie Ende des U-Rohrs mit einem geeigneten Aufnahmetank verbindbar ist.

32. Gruppe nach einem der Ansprüche 22 bis 31, **dadurch gekennzeichnet, dass** das Lufteinlassmittel jeder Vorrichtung mit einem gemeinsamen Lufteinlassrohrsystem mit einem freien Ende, das daran optional geeignete aseptische Verbindungsmittel umfasst, geeignet verbunden ist.

33. Gruppe nach Anspruch 32, **dadurch gekennzeichnet, dass** das freie Ende mit einer geeigneten Luftzufuhr verbindbar ist.

34. Verfahren für das axenischen Kultivieren und Ernten von Zellen und/oder Gewebe in einer Einwegvorrichtung, welches folgende Schritte umfasst:

a) Vorsehen der Vorrichtung, welche einen sterilisierbaren transparenten und/oder lichtdurchlässigen nicht starren Einwegbehälter mit einem oberen Ende und einem unteren Ende umfasst, welcher zumindest teilweise mit einem geeigneten sterilen biologischen Zell- und/oder Gewebekulturmedium und/oder axenischen Inokulant und/oder steriler Luft und/oder erforderlichen anderen sterilen Zusätzen gefüllt werden kann, wobei der Behälter Folgendes umfasst:

(i) Gasauslassmittel für das Ablassen überschüssiger Luft und/oder Abgase aus dem Behälter;

(ii) Zusatzeinlassmittel für das Einbringen des Inokulants und/oder des Kulturmediums und/oder der Zusätze in den Behälter; und **dadurch gekennzeichnet, dass** er weiterhin umfasst:

(iii) wiederverwendbare Erntemittel, welche geeignete Stromsteuermittel für das Ermöglichen des Erntens mindestens eines gewünschten Teils des die Zellen und/oder das Gewebe enthaltenden Mediums, wenn dies gewünscht ist, umfassen, wodurch die Vorrichtung ständig für mindestens einen weiteren folgenden Kultivier-/Erntezyklus verwendet werden kann, wobei der Behälter so ausgelegt ist, dass ein Rest des die Zellen und/oder das Gewebe enthaltenden Mediums in dem Behälter verbleiben kann, um als Inokulant für einen nächsten Kultur- und Erntezyklus zu dienen;

b) Zuführen eines axenischen Inokulants über das Erntemittel;

c) Zuführen eines sterilen Kulturmediums und/oder steriler Zusätze über das Zusatzeinlassmittel;

d) Optionales Beleuchten des Behälters mit einem externen Beleuchtungsmittel;

e) Wachsenlassen der Zellen und/oder des Gewebes in dem Medium auf eine gewünschte Ausbeute;

f) Kontinuierliches Ablassen überschüssiger Luft und/oder Abgase aus dem Behälter über das Gasauslassmittel;

g) Prüfen auf Verunreinigungen und/oder der Qualität der Zellen/Gewebe, die in dem Behälter erzeugt werden: wenn Verunreinigungen gefunden werden oder die erzeugten Zellen/Gewebe von schlechter Qualität sind, werden die Vorrichtung und ihr Inhalt entsorgt; wenn keine Verunreinigungen gefunden werden, wird Schritt h) durchgeführt;

h) Ernten des gewünschten Teils des die Zellen und/oder das Gewebe enthaltenden Mediums, während der Rest des die Zellen und/oder das Gewebe enthaltenden Mediums in dem Behälter belassen wird, wobei der Rest des Mediums als Inokulant für einen nächsten Kultur-/Erntezyklus dient;

i) Zuführen eines sterilen Kulturmediums und/oder steriler Zusätze für den nächsten Kultur-/Erntezyklus über das Zusatzeinlassmittel;

j) Mehrfaches Wiederholen der Schritte d), e), f), g), h) und i) bis bei g) Verunreinigungen gefunden werden oder die erzeugten Zellen/Gewebe von schlechter Qualität sind, woraufhin die Vorrichtung und ihr Inhalt entsorgt werden.

35. Verfahren nach Anspruch 34, **dadurch gekennzeichnet, dass** die Vorrichtung weiterhin Lufteinlassmittel für das Einleiten steriler Luft in Form von Bläschen in das Kulturmedium durch eine erste Einlassöffnung, die mit einer geeigneten sterilen Luftzufuhr verbindbar ist, umfasst, wobei das Verfahren weiterhin den Schritt des Zuführens steriler Luft zu dem Lufteinlassmittel während des ersten und jedes folgenden Zyklus umfasst.

36. Verfahren nach Anspruch 35, **dadurch gekennzeichnet, dass** die sterile Luft während mindestens eines Kultivierzyklus ständig zugeführt wird.

37. Verfahren nach Anspruch 35, **dadurch gekennzeichnet, dass** die sterile Luft während mindestens eines Kultivierzyklus stoßweise zugeführt wird.

38. Verfahren für das axenische Kultivieren und Ernten von Zellen und/oder Gewebe in einer Gruppe von Einwegvorrichtungen, welches folgende Schritte umfasst:

a) Vorsehen einer Gruppe von Vorrichtungen nach Anspruch 30 und für mindestens eine Vorrichtung derselben:

b) Zuführen eines axenischen Inokulants zur Vorrichtung über das gemeinsame Ernterohrsystem;

c) Zuführen eines sterilen Kulturmediums und/oder steriler Zusätze zur Vorrichtung über das gemeinsame Zusatzeinlassrohrsystem;

d) Optionales Beleuchten des Behälters mit einem externen Beleuchtungsmittel;

e) Wachsenlassen der Zellen und/oder des Gewebes in der Vorrichtung in dem Medium auf eine gewünschte Ausbeute;

f) Kontinuierliches Ablassen überschüssiger Luft und/oder Abgase aus der Vorrichtung über das gemeinsame Gasauslassrohrsystem;

g) Prüfen auf Verunreinigungen und/oder der Qualität der Zellen/Gewebe, die in der Vorrichtung erzeugt werden: wenn Verunreinigungen in der Vorrichtung gefunden werden oder die erzeugten Zellen/Gewebe von schlechter Qualität sind, wird das Erntemittel der Vorrichtung abgesperrt, was eine Verunreinigung der anderen Vorrichtungen der Gruppe verhindert; wenn in allen Vorrichtungen der Gruppe Verunreinigungen gefunden werden oder die darin erzeugten Zellen/Gewebe von schlechter Qualität sind, werden alle Vorrichtungen und deren Inhalt entsorgt; wenn keine Verunreinigungen gefunden werden und die Qualität der erzeugten Zellen/Gewebe annehmbar ist, wird die Vorrichtung als erntefähig betrachtet und Schritt h) wird durchgeführt;

h) bei jeder erntefähigen Vorrichtung von Schritt g) Ernten des gewünschten Teils des die Zellen und/oder das Gewebe enthaltenden Mediums über das gemeinsame Ernterohrsystem und das Verunreinigungsverhinderungsmittel zu einem geeigneten Aufnahmetank, während der Rest des die Zellen und/oder das Gewebe enthaltenden Mediums in dem Behälter belassen wird, wobei der zweite Teil des Mediums als Inokulant für einen nächsten Kultur-/Erntezyklus dient;

i) Zuführen eines sterilen Kulturmediums und/oder steriler Zusätze für den nächsten Kultur-/Erntezyklus über das Zusatzeinlassmittel;

j) Mehrfaches Wiederholen der Schritte d), e), f), g), h) und i) bis bei g) bei allen Vorrichtungen der Gruppe Verunreinigungen gefunden werden oder die erzeugten Zellen/Gewebe von schlechter Qualität sind, woraufhin die Verunreinigungsverhinderungsmittel von dem gemeinsamen Erntemittel getrennt werden und die Vorrichtungen und ihr Inhalt entsorgt werden.

39. Verfahren für das axenische Kultivieren und Ernten von Zellen und/oder Gewebe in einer Gruppe von Einwegvorrichtungen, welches folgende Schritte umfasst:

a) Vorsehen einer Gruppe von Vorrichtungen nach Anspruch 33 und für mindestens eine Vorrichtung derselben:

b) Zuführen eines axenischen Inokulants zur Vorrichtung über das gemeinsame Ernterohrsystem;

c) Zuführen eines sterilen Kulturmediums und/oder steriler Zusätze zur Vorrichtung über das gemeinsame Zusatzeinlassrohrsystem;

d) Zuführen steriler Luft zur Vorrichtung über das gemeinsame Lufteinlassrohrsystem;

e) Optionales Beleuchten des Behälters mit einem externen Beleuchtungsmittel;

f) Wachsenlassen der Zellen und/oder des Gewebes in der Vorrichtung in dem Medium auf eine gewünschte Ausbeute;

g) Kontinuierliches Ablassen überschüssiger Luft und/oder Abgase aus der Vorrichtung über das gemeinsame Gasauslassrohrsystem;

h) Prüfen auf Verunreinigungen und/oder der Qualität der Zellen/Gewebe, die in der Vorrichtung erzeugt werden: wenn Verunreinigungen in der Vorrichtung gefunden werden oder die erzeugten Zellen/Gewebe von schlechter Qualität sind, wird das Erntemittel der Vorrichtung abgesperrt, was eine Verunreinigung der anderen Vorrichtungen der Gruppe verhindert; wenn in allen Vorrichtungen der Gruppe Verunreinigungen gefunden werden oder die darin erzeugten Zellen/Gewebe von schlechter Qualität sind, werden alle Vorrichtungen und deren Inhalt entsorgt; wenn keine Verunreinigungen gefunden werden und die Qualität der erzeugten Zellen/Gewebe annehmbar ist, wird die Vorrichtung als erntefähig betrachtet und Schritt i) wird durchgeführt;

i) bei jeder erntefähigen Vorrichtung von Schritt h) Ernten mindestens eines gewünschten Teils des die Zellen und/oder das Gewebe enthaltenden Mediums über das gemeinsame Ernterohrsystem und das Verunreinigungsverhinderungsmittel zu einem geeigneten Aufnahmetank, während der Rest des die Zellen und/oder das Gewebe enthaltenden Mediums in dem Behälter belassen wird, wobei der Rest des Mediums als Inokulant für einen nächsten Kultur-/Erntezyklus dient;

j) Zuführen eines sterilen Kulturmediums und/oder steriler Zusätze für den nächsten Kultur-/Erntezyklus über das Zusatzeinlassmittel;

k) Mehrfaches Wiederholen der Schritte d), e), f), g), h), i) und j) bis bei h) bei allen Vorrichtungen der Gruppe die Verunreinigungen gefunden werden oder die erzeugten Zellen/Gewebe von schlechter Qualität sind, woraufhin die Verunreinigungsverhinderungsmittel von dem gemeinsamen Erntemittel getrennt werden und die Vorrichtungen und ihr Inhalt entsorgt werden.

Revendications

1. Dispositif jetable (10) pour cultiver et collecter de manière axénique des cellules et/ou un tissu sur au moins un cycle, ledit dispositif comportant un conteneur non-rigide jetable (20) transparent et/ou translucide stérilisable ayant une extrémité supérieure (26) et une extrémité inférieure (28), lequel conteneur peut être au moins partiellement rempli d'un milieu de culture stérile adapté de cellules biologiques et/ou tissus et/ou d'un agent d'inoculation axénique et/ou d'air stérile et/ou d'autres additifs stériles nécessaires, ledit conteneur comportant :

   (i) des moyens de sortie de gaz (90, 96) pour éliminer de l'air et/ou des gaz usés en excès depuis ledit conteneur,
   (ii) des moyens d'entrée d'additifs (80, 86) pour introduire ledit agent d'inoculation et/ou ledit milieu de culture et/ou lesdits additifs dans ledit conteneur,
   et **caractérisé en ce qu'**il comporte de plus
   (iii) de moyens de collecte réutilisables (50, 52, 56) comportant des moyens de commande d'écoulement adaptés (54, 55) pour permettre la collecte d'au moins une partie voulue dudit milieu contenant des cellules et/ou des tissus lorsqu'on le souhaite, de manière à permettre audit dispositif d'être utilisé en continu pendant au moins un cycle de culture/collecte consécutif supplémentaire,

   dans lequel ledit conteneur est adapté (d2) pour permettre au reste dudit milieu contenant des cellules et/ou un tissu de rester dans ledit conteneur pour servir en tant que d'agent d'inoculation pour un cycle de culture et de collecte suivant.

2. Dispositif selon la revendication 1, comportant de plus des moyens d'entrée d'air pour introduire de l'air stérile sous la forme de bulles dans ledit milieu de culture à travers une première ouverture d'entrée, dans lequel lesdits moyens d'entrée d'air peuvent être connectés à une alimentation en air adaptée.

3. Dispositif selon la revendication 1 ou 2, dans lequel lesdits moyens de collecte comportent des moyens de prévention ou de contamination pour sensiblement empêcher l'introduction de contaminants dans ledit conteneur via lesdits moyens de collecte.

4. Dispositif selon l'une quelconque des revendications précédentes, dans lequel ledit conteneur est constitué d'un matériau plastique non-rigide.

5. Dispositif selon la revendication 4, dans lequel ledit matériau est sélectionné parmi le groupe constitué de polyéthylène, de polycarbonate, d'un copolymère de polyéthylène et de Nylon, de PVC et de EVA.

6. Dispositif selon la revendication 4 ou 5, dans lequel ledit conteneur est constitué d'un stratifié de plus d'une couche desdits matériaux.

7. Dispositif selon l'une quelconque des revendications 4 à 6, dans lequel ledit conteneur est formé en

reliant par fusion deux feuilles adaptées dudit matériau le long de jonctions prédéterminées.

8. Dispositif selon l'une quelconque des revendications précédentes, dans lequel lesdits moyens d'entrée d'air comportent un tuyau d'entrée d'air s'étendant depuis ladite ouverture d'entrée jusqu'à un emplacement à l'intérieur dudit conteneur sur ladite extrémité inférieure de celui-ci, ou à proximité de celle-ci.

9. Dispositif selon l'une quelconque des revendications précédentes, dans lequel au moins une partie desdites bulles d'air ont un diamètre moyen compris entre environ 1 mm et environ 10 mm.

10. Dispositif selon l'une quelconque des revendications précédentes, dans lequel au moins une partie desdites bulles d'air ont un diamètre moyen d'environ 4 mm.

11. Dispositif selon l'une quelconque des revendications précédentes, dans lequel ledit conteneur comporte un filtre adapté monté sur lesdits moyens de sortie de gaz pour sensiblement empêcher l'introduction de contaminants dans ledit conteneur via lesdits moyens de sortie de gaz.

12. Dispositif selon l'une quelconque des revendications précédentes, dans lequel ledit conteneur comporte de plus un filtre adapté monté sur lesdits moyens d'entrée d'additifs pour sensiblement empêcher l'introduction de contaminants dans ledit conteneur via lesdits moyens d'entrée d'additifs.

13. Dispositif selon l'une quelconque des revendications précédentes, dans lequel lesdits moyens de prévention de contamination comportent un piège à fluide en forme de U, dans lequel un bras de celui-ci est monté de manière aseptique sur une sortie externe desdits moyens de collecte par des moyens de connexion aseptique adaptés.

14. Dispositif selon l'une quelconque des revendications précédentes, dans lequel lesdits moyens de collecte sont situés sur la partie inférieure de ladite extrémité inférieure dudit conteneur.

15. Dispositif selon l'une quelconque des revendications 1 à 13, dans lequel lesdits moyens de collecte sont situés à proximité de la partie inférieure de ladite extrémité inférieure dudit conteneur, de sorte que, à la fin de chaque oyats de collecte, ledit reste dudit milieu contenant des cellules et/ou un tissu reste automatiquement dans ladite extrémité inférieure dudit conteneur jusqu'à un niveau situé au-dessous du niveau desdits moyens de collecte.

16. Dispositif selon l'une quelconque des revendications précédentes, dans lequel ledit reste dudit milieu contenant des cellules et/ou un tissu comporte entre environ 2,5 % et environ 45 %, et de préférence entre environ 10 % et environ 20 % du volume d'origine dudit milieu de culture et dudit agent d'inoculation.

17. Dispositif selon l'une quelconque des revendications précédentes, dans lequel ladite extrémité inférieure est sensiblement convexe.

18. Dispositif selon l'une quelconque des revendications 1 à 16, dans lequel ladite extrémité inférieure est sensiblement tronconique.

19. Dispositif selon l'une quelconque des revendications précédentes, dans lequel ledit conteneur comporte un volume remplissable interne compris entre environ 5 litres et environ 50 litres.

20. Dispositif selon l'une quelconque des revendications précédentes, dans lequel ledit dispositif comporte de plus des moyens de fixation adaptés pour fixer celui-ci à une structure de support adaptée.

21. Dispositif selon la revendication 20, dans lequel lesdits moyens de fixation comportent une boucle de matériau fixée de préférence en un seul bloc à ladite extrémité supérieure dudit conteneur.

22. Batterie desdits dispositifs, comportant au moins deux desdits dispositifs jetables selon l'une quelconque des revendications précédentes.

23. Batterie selon la revendication 22, dans laquelle lesdits dispositifs sont supportés par une structure de support adaptée via lesdits moyens de fixation de chacun desdits dispositifs.

24. Batterie selon les revendications 22 ou 23, dans laquelle lesdits moyens de sortie de gaz de chacun desdits dispositifs sont connectés de manière adaptée à une canalisation de sortie de gaz commune qui comporte de manière facultative des moyens adaptés pour empêcher que des contaminants ne s'écoulent dans lesdits dispositifs.

25. Batterie selon la revendication 24, dans laquelle lesdits moyens pour empêcher l'écoulement de contaminants dans lesdits dispositifs comportent un filtre adapté.

26. Batterie selon l'une quelconque des revendications 22 à 25, dans laquelle lesdits moyens d'entrée d'additifs de chacun desdits dispositifs sont connectés de manière adaptée à une canalisation d'entrée d'additifs commune ayant une extrémité libre com-

portant de manière facultative des moyens de connexion aseptique adaptés sur celle-ci.

27. Batterie selon la revendication 26, dans laquelle ladite extrémité libre peut être connectée à une alimentation adaptée de milieu et/ou additifs.

28. Batterie selon l'une quelconque des revendications 22 à 27, dans laquelle lesdits moyens de collecte de chacun desdits dispositifs sont connectés de manière adaptée à une canalisation de collecte commune ayant une extrémité libre comportant de manière facultative des moyens de connexion aseptique adaptés sur celle-ci.

29. Batterie selon la revendication 28, comportant de plus des moyens de prévention de contamination pour sensiblement empêcher l'introduction de contaminants dans ledit conteneur via ladite canalisation de collecte commune.

30. Batterie selon la revendication 21, dans laquelle lesdits moyens de prévention de contamination comportent un piège à fluide en forme de U, dans lequel un bras de celui-ci est libre ayant une ouverture et dans lequel l'autre extrémité de celui-ci peut être montée de manière aseptique sur ladite extrémité libre de ladite canalisation de collecte commune via des moyens de connexion aseptique adaptés.

31. Batterie selon la revendication 30, dans laquelle ladite extrémité libre dudit tube en forme de U peut être connectée à un réservoir de réception adapté.

32. Batterie selon l'une quelconque des revendications 22 à 31, dans laquelle lesdits moyens d'entrée d'air de chacun desdits dispositifs sont connectés de manière adaptée à une canalisation d'entrée d'air commune ayant une extrémité libre comportant de manière facultative des moyens de connexion aseptique adaptés sur celle-ci.

33. Batterie selon la revendication 32, dans laquelle ladite extrémité libre peut être connectée à une alimentation en air adaptée.

34. Procédé pour cultiver et collecter de manière axénique des cellules et/ou un tissu dans un dispositif jetable comportant les étapes consistant à :

a) fournir ledit dispositif qui comporte un conteneur non-rigide jetable transparent et/ou translucide stérilisable ayant une extrémité supérieure et une extrémité inférieure, lequel conteneur peut être au moins partiellement rempli d'un milieu de culture stérile adapté de cellules biologiques et/ou tissus et/ou d'un agent d'inoculation axénique et/ou d'air stérile et/ou d'autres additifs stériles nécessaires, ledit conteneur comportant :

(i) des moyens de sortie de gaz pour éliminer de l'air et/ou des gaz usés en excès depuis ledit conteneur,
(ii) des moyens d'entrée d'additifs pour introduire ledit agent d'inoculation et/ou ledit milieu de culture et/ou lesdits additifs dans ledit conteneur,
et **caractérisé en ce qu'il** comporte de plus :
(iii) des moyens de collecte réutilisables comportant des moyens de commande d'écoulement adaptés pour permettre la collecte d'au moins une partie voulue dudit milieu contenant des cellules et/ou un tissu lorsqu'on le souhaite, de manière à permettre audit dispositif d'être utilisé en continu sur au moins un cycle de culture/collecte consécutif supplémentaire, dans lequel ledit conteneur est adapté pour permettre au reste dudit milieu contenant des cellules et/ou un tissu de rester dans ledit conteneur pour servir en tant qu'agent d'inoculation pour un cycle de culture et de collecte suivant,

b) alimenter un agent d'inoculation axénique via lesdits moyens de collecte,
c) alimenter ledit milieu de culture stérile et/ou lesdits additifs stériles via lesdits moyens d'entrée d'additifs,
d) illuminer de manière facultative ledit conteneur à l'aide de moyens lumineux externes,
e) permettre auxdites cellules et/ou audit tissu de croître dans ledit milieu jusqu'à un rendement voulu,
f) permettre à de l'air et/ou des gaz usés en excès de quitter le conteneur en continu via lesdits moyens de sortie de gaz,
g) contrôler des contaminants et/ou la qualité des cellules/tissus qui sont produits dans ledit conteneur, si des contaminants sont trouvés où les cellules/tissus qui sont produits sont d'une faible qualité, le dispositif et son contenu sont mis au rebut, si des contaminants ne sont pas trouvés, l'étape h) est exécutée,
h) collecter ladite partie voulue dudit milieu contenant des cellules et/ou un tissu, tout en laissant ledit reste de milieu contenant des cellules et/ou un tissu dans ledit conteneur, ledit reste de milieu servant en tant qu'agent d'inoculation pour un cycle de culture/collecte suivant,
i) alimenter ledit milieu de culture stérile et/ou lesdits additifs stériles pour le cycle de culture/collecte suivant via lesdits moyens d'entrée

d'additifs,
j) répéter les étapes d), e), f), g), h) et i) une pluralité de fois jusqu'à ce que, à l'étape g), lesdits contaminants soient trouvés ou les cellules/tissus qui sont produits soient de faible qualité, après quoi le dispositif et son contenu sont mis au rebut.

35. Procédé selon la revendication 39, dans lequel ledit dispositif comporte de plus des moyens d'entrée d'air pour introduire de l'air stérile sous la forme de bulles dans ledit milieu de culture à travers une première ouverture d'entrée pouvant être connectés à une alimentation en air stérile adaptée, ledit procédé comportant de plus l'étape consistant à alimenter de l'air stérile dans lesdits moyens d'entrée d'air pendant le premier cycle et chaque cycle ultérieur.

36. Procédé selon la revendication 35, dans lequel ledit air stérile est alimenté en continu tout au long d'au moins un cycle de culture.

37. Procédé selon la revendication 35, dans lequel ledit air stérile est alimenté sous forme d'impulsions pendant au moins un cycle de culture.

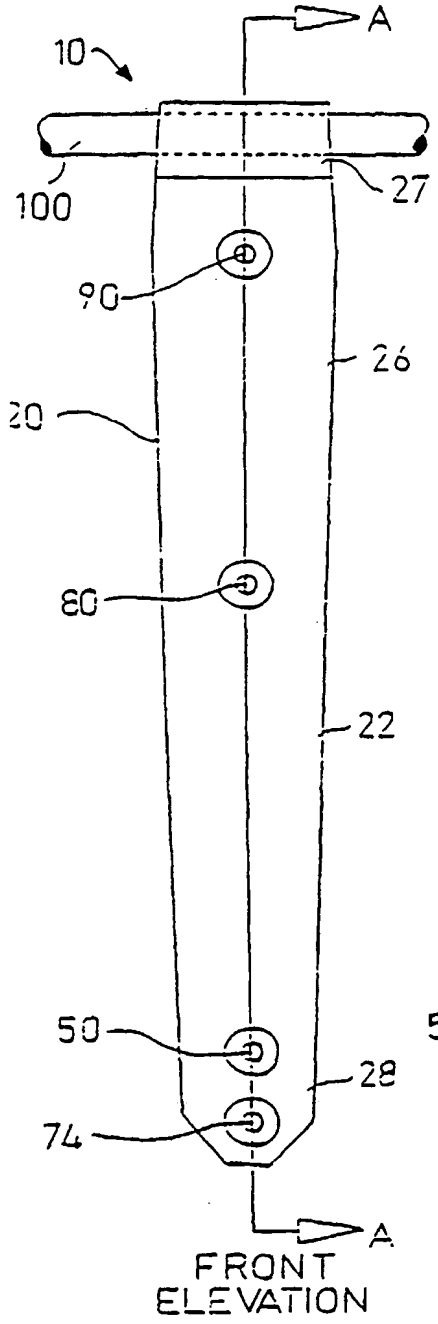
38. Procédé pour cultiver et collecter de manière axénique des cellules et/ou un tissu dans une batterie de dispositifs jetables comportant les étapes consistant à :

a) fournir une batterie de dispositifs selon la revendication 30, et pour au moins un dispositif de celle-ci,
b) alimenter un agent d'inoculation axénique dans ledit dispositif via ladite canalisation de collecte commune,
c) alimenter ledit milieu de culture stérile et/ou lesdits additifs stériles dans ledit dispositif via ladite canalisation d'entrée d'additifs commune,
d) illuminer de manière facultative ledit dispositif à l'aide de moyens lumineux externes,
e) permettre auxdites cellules et/ou audit tissu dudit dispositif de croître dans ledit milieu jusqu'à un rendement voulu,
f) permettre à de l'air et/ou des gaz usés en excès de quitter ledit dispositif en continu via ladite canalisation de sortie de gaz commune,
g) contrôler des contaminants et/ou la qualité des cellules/tissus qui sont produits dans ledit dispositif, si dans ledit dispositif des contaminants sont trouvés où si les cellules/tissus qui sont produits sont de faible qualité, lesdits moyens de collecte dudit dispositif sont fermés empêchant une contamination desdits autres dispositifs de ladite batterie, si dans la totalité desdits dispositifs de ladite batterie des contaminants sont trouvés ou les cellules/tissus qui sont produits à l'intérieur sont d'une faible qualité, tous les dispositifs et leurs contenus sont mis au rebut, si des contaminants ne sont trouvés et la qualité des cellules/tissus produits est acceptable, le dispositif est considéré comme étant utilisable pour une collecte et l'étape h) est exécutée,
h) pour chacun desdits dispositifs utilisables pour une collecte de l'étape g), collecter ladite partie voulue dudit milieu contenant des cellules et/ou un tissu via ladite canalisation de collecte commune et lesdits moyens de prévention de contamination dans un réservoir de réception adapté, tout en laissant ledit reste de milieu contenant des cellules et/ou un tissu dans ledit conteneur, dans lequel ladite seconde partie de milieu sert en tant qu'agent d'inoculation pour un cycle de culture/collecte suivant,
i) alimenter ledit milieu de culture stérile et/ou lesdits additifs stériles pour le cycle de culture/collecte suivant via lesdits moyens d'entrée d'additifs,
j) répéter les étapes d), e), f), g), h) et i) une pluralité de fois jusqu'à ce que, à l'étape g), lesdits contaminants soient trouvés ou les cellules/tissus qui sont produits soient de faible qualité pour tous lesdits dispositifs de ladite batterie, après quoi lesdits moyens de prévention de contamination sont déconnectés desdits moyens de collecte communs et lesdits dispositifs et leurs contenus sont mis au rebut.

39. Procédé pour cultiver et collecter de manière axénique des cellules et/ou un tissu dans une batterie de dispositifs jetables comportant les étapes consistant à :

a) fournir une batterie de dispositifs selon la revendication 33, et pour au moins un dispositif de celle-ci,
b) alimenter un agent d'inoculation axénique dans ledit dispositif via ladite canalisation de collecte commune,
c) alimenter ledit milieu de culture stérile et/ou lesdits additifs stériles dans ledit dispositif via ladite canalisation d'entrée d'additifs commune,
d) alimenter de l'air stérile dans ledit dispositif via ladite canalisation d'entrée d'air commune,
e) illuminer de manière facultative ledit dispositif à l'aide de moyens lumineux externes,
f) permettre auxdites cellules et/ou audit tissu dudit dispositif de croître dans ledit milieu jusqu'à un rendement voulu,
g) permettre à de l'air et/ou des gaz usés en excès de quitter ledit dispositif en continu via

ladite canalisation de sortie de gaz commune,
h) contrôler des contaminants et/ou la qualité des cellules/tissus qui sont produits dans ledit dispositif, si dans ledit dispositif des contaminants sont trouvés où si les cellules/tissus qui sont produits sont de faible qualité, lesdits moyens de collecte dudit dispositif sont fermés empêchant une contamination desdits autres dispositifs de ladite batterie, si dans la totalité desdits dispositifs de ladite batterie des contaminants sont trouvés ou les cellules/tissus qui sont produits à l'intérieur sont d'une faible qualité, tous les dispositifs et leurs contenus sont mis au rebut, si des contaminants ne sont trouvés et la qualité des cellules/tissus produits est acceptable, le dispositif est considéré comme étant utilisable pour une collecte et l'étape i) est exécutée,
i) pour chacun desdits dispositifs utilisables pour une collecte de l'étap h), collecter au moins une partie voulue dudit milieu contenant des cellules et/ou un tissu via ladite canalisation de collecte commune et lesdits moyens de prévention de contamination dans un réservoir de réception adapté, tout en laissant ledit reste de milieu contenant des cellules et/ou un tissu dans ledit conteneur, dans lequel ledit reste de milieu sert en tant qu'agent d'inoculation pour un cycle de culture/collecte suivant,
j) alimenter ledit milieu de culture stérile et/ou lesdits additifs stériles pour le cycle de culture/collecte suivant via lesdits moyens d'entrée d'additifs,
k) répéter les étapes d), e), f), g), h), i) et j) une pluralité de fois jusqu'à ce que, à l'étape h), lesdits contaminants soient trouvés ou les cellules/tissus qui sont produits soient de faible qualité pour tous lesdits dispositifs de ladite batterie, après quoi lesdits moyens de prévention de contamination sont déconnectés desdits moyens de collecte communs et lesdits dispositifs et leurs contenus sont mis au rebut.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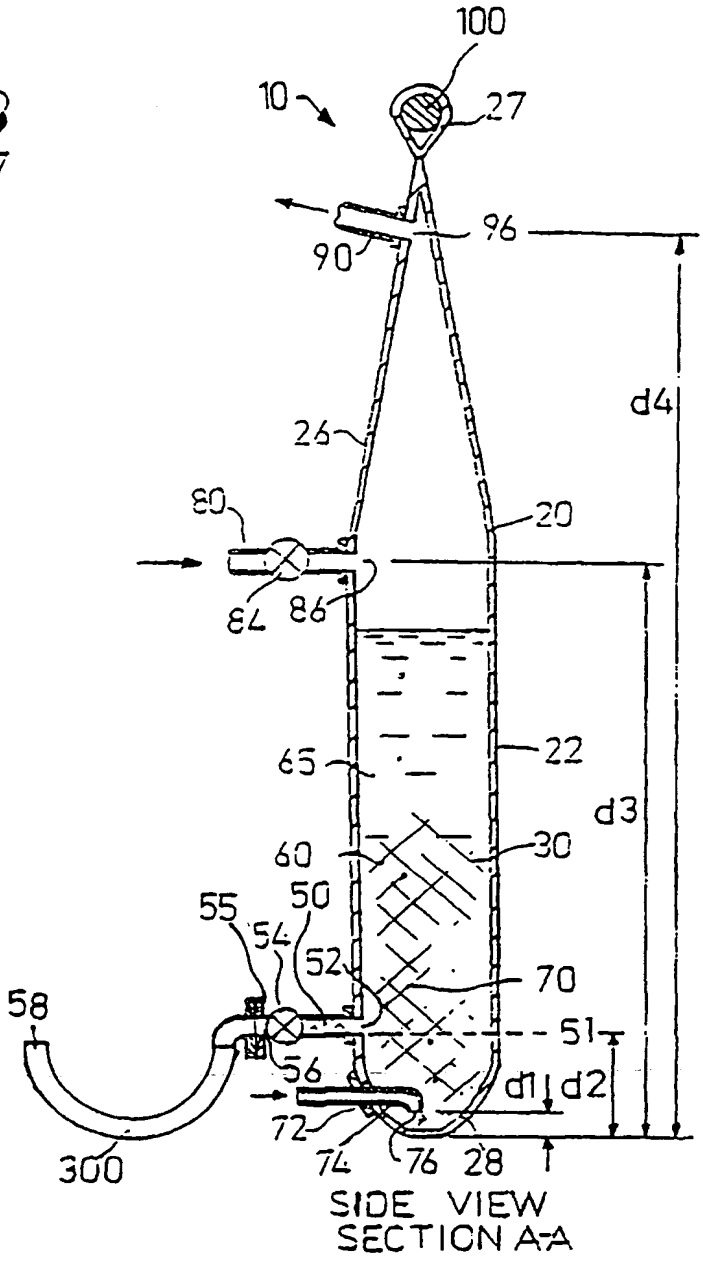
A
FRONT
ELEVATION

Fig. 1a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30
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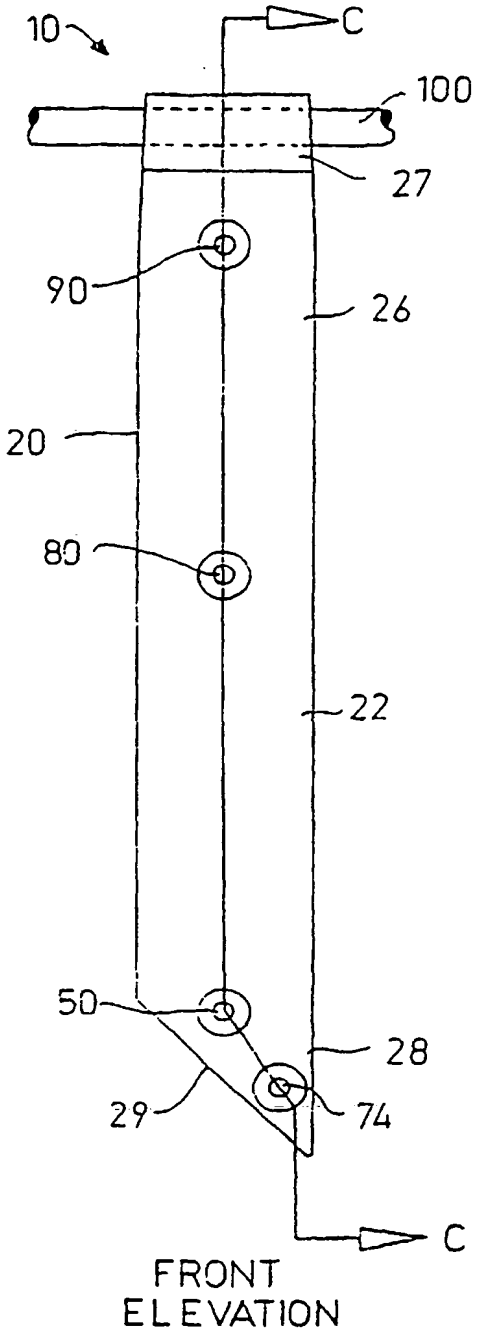
58
51
56
d1
d2
72
74
76
28
300
SIDE VIEW
SECTION A-A

Fig. 1b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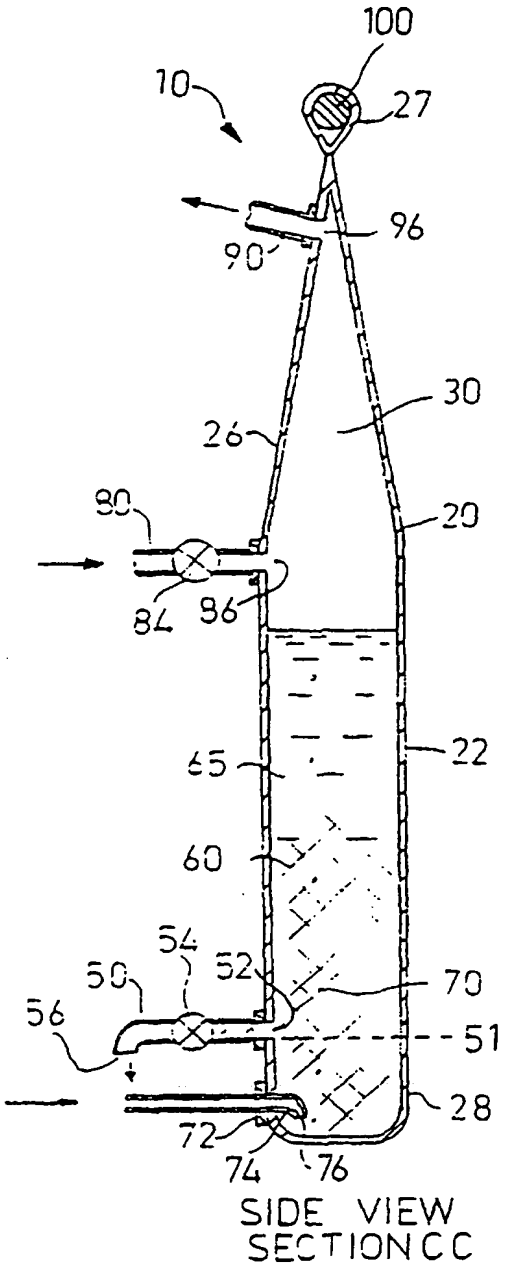
ELEVATION

Fig. 2a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SIDE VIEW
SECTION C C

Fig. 2b

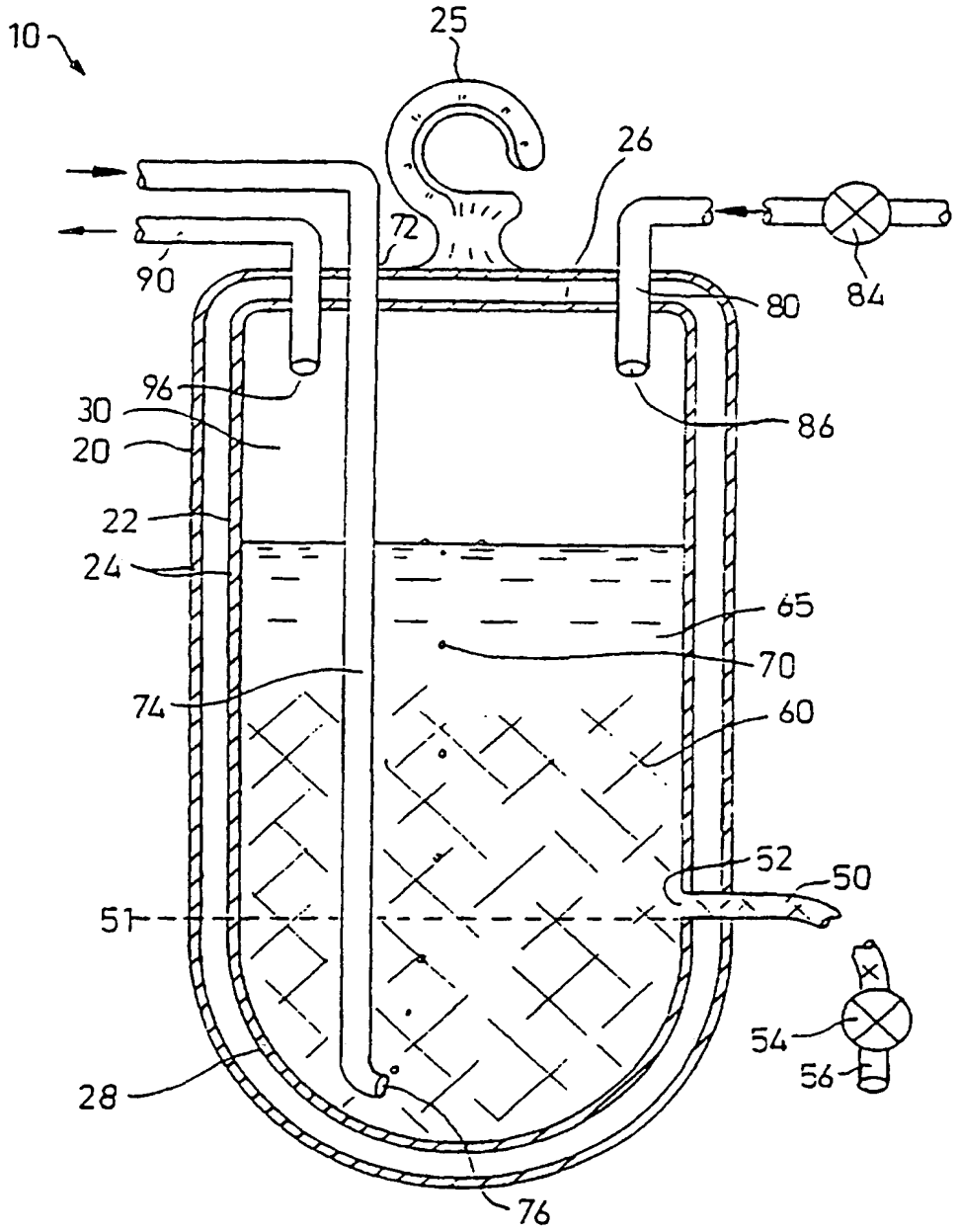


Fig. 3

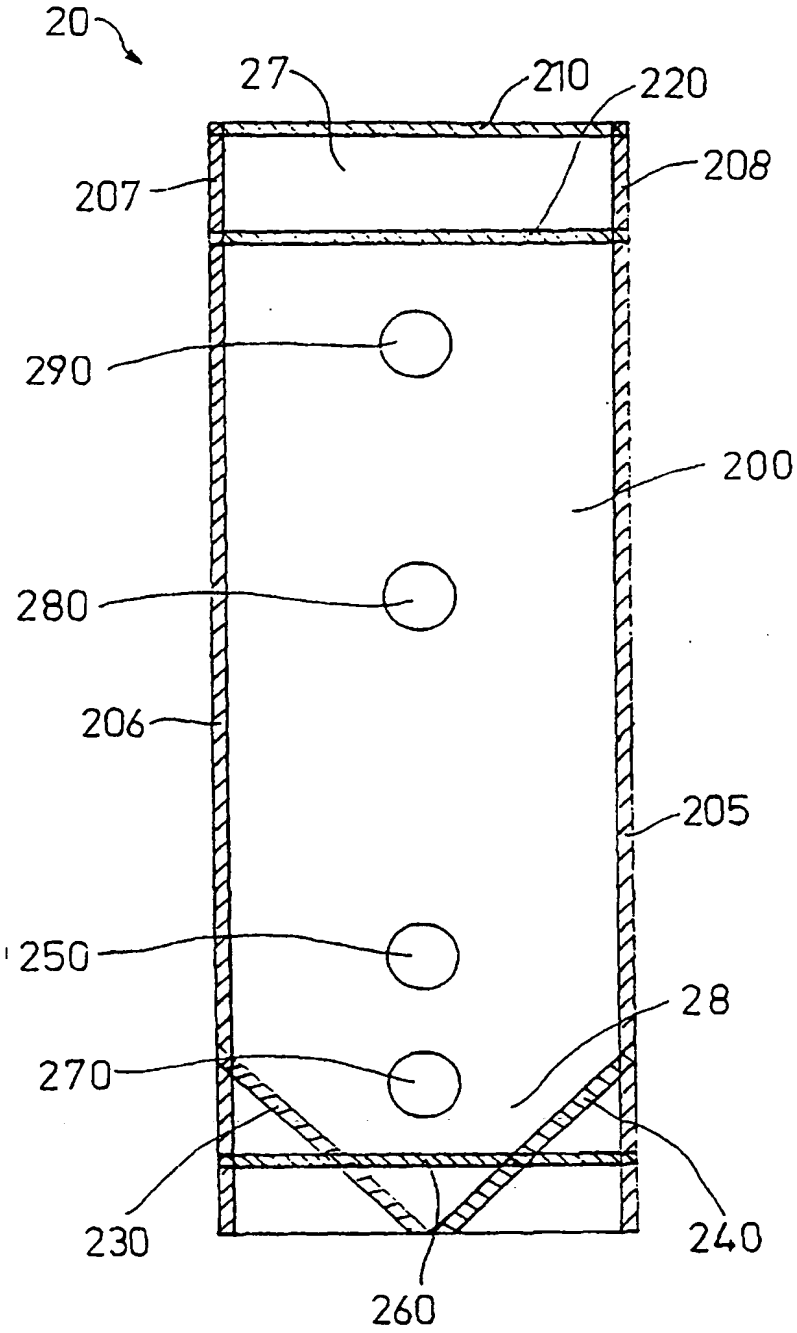
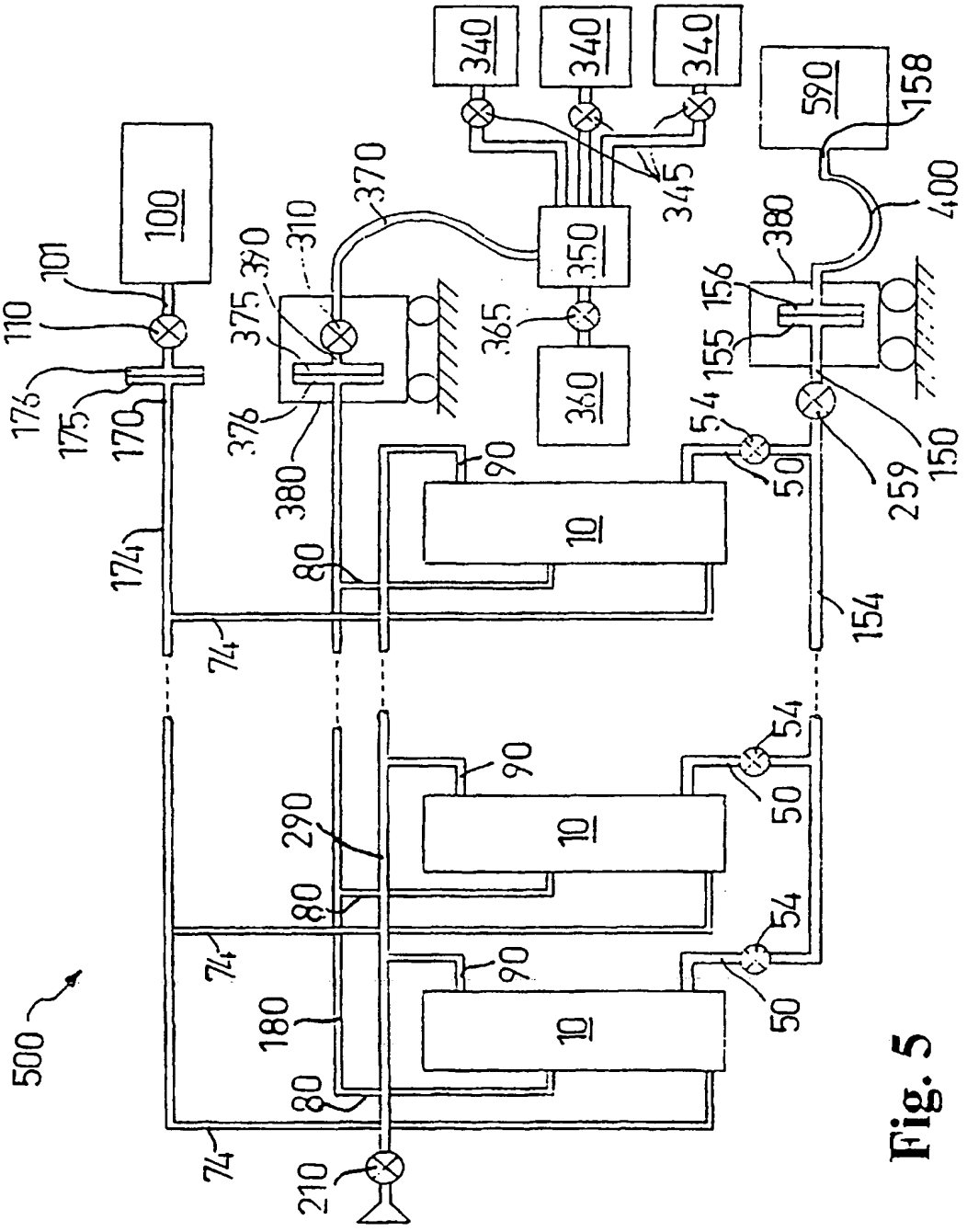


Fig. 4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259
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500

Fig. 5